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PHYSICO-CHEMICAL FACTORS INFLUENCING CREAM RISING

I. VISCOSITY*

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The viscosity of milk has been considered (1) to be a factor influencing cream rising, but with the exception of the studies by van Dam and Sirks (2) and by Rahn (3) no data have been published attempting to correlate the viscosity with differences in the depth of the cream layer.

In the study by van Dam various substances (e.g., gum tragacanth, gelatin, starch, gum arabic, agar, and saleb, a material obtained by boiling Irish moss in linseed oil) were added to milk in suitable amounts so that in most cases there was little or no change in specific gravity. Gum tragacanth and saleb gave in general the best results. These substances caused a 15 to 25 per cent increase in the depth of the cream layer at 9°C. which was accompanied by an increase in viscosity of 25 per cent showing that with added substances there is some relation between viscosity and cream rising. Van Dam and Sirks have expressed the opinion that it is the milk plasma, not the fat, which has the chief influence on cream rising.

In Rahn's study gelatin and other hydrophilic colloids when added to milk increased both the cream layer and the viscosity in fairly close ratio. For example, the addition of 0.45, 1.35, and 1.8 per cent gelatin increased the viscosity 14.7, 67.0, and 110 per cent respectively with a corresponding increase of 33, 55, and 83 per cent in the depth of the cream layer. Although

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Rahn has concluded that variations in creaming are not due to variations in viscosity, his own data hardly warrant this view.

The viscosity of milk is due to the combined effect of the constituents in true and in colloidal solution together with the emulsified fat. If one compares the viscosity of whey, skim milk, and whole milk with that of water, it is seen that water contributes from 30 to 50 per cent of the viscosity. Of the increase due to the milk solids, about 30 to 40 per cent is caused by the constituents in the whey, 70 to 90 per cent by the constituents in the skim milk and the remainder by the fat. The important fact is that most of the specific viscosity of milk is due to the solids-not-fat. Kobler (4) in fact, from whose data the above estimations are largely made, concluded that the viscosity of milk is greatly influenced by the casein.

There are a number of statements in the literature relative to the importance of the solids-not-fat in cream rising. Babcock (1) and Hunziker (5) have expressed such a view, the latter believing that the reduced cream volume usually found on pasteurized milk is due to a coagulation of the albumin.

A careful consideration of the whole subject of cream rising leads to the conclusion that the two outstanding factors involved in determining the volume of cream rising as a definite layer on a given lot of milk are, (a) the rapidity and completeness with which the fat globules rise, (b) the volume occupied by the risen fat globules. The fat globules rise in milk fundamentally because of the difference between the specific gravity of the fat and the plasma. Although the size of the fat drops and the extent of their association (3) no doubt contribute a great deal to the rapidity and exhaustiveness of creaming in a given length of time, they can not be the only factors involved. It is certain that there is as yet no basis for assuming that either the size of the fat globules or their clumping determines the volume occupied by the risen fat. In making these assertions conditions involving a uniform fat content are of course assumed.

The present study was undertaken to determine the extent to which the viscosity of milk is related to the depth of the cream layer on both raw and pasteurized milk. The data secured also

throw light on the relative importance of viscosity and size of fat globules in cream rising as met under normal conditions. Of special interest are the results showing the distribution of fat between the cream and skim milk layers.

Methods of procedure

The milk used was secured from the University herd. Uniform quality was approached as nearly as possible by using milk from the same cows in all experiments where mixed milk was used. In these cases the milk represented two-thirds Holstein milk and one-third Jersey-Guernsey. For the breed experiments, milk from the Jerseys and Guernseys represented one standard and milk from Holsteins the other. It was not feasible to control any possible effect of the stage of lactation. This effect could not have been significant, however, because a comparison of the viscosity of the milk and stage of lactation of 8 cows, 4 Holstein and 4 Guernsey, whose lactation period ranged from six days to seven hundred fifty-one days, showed no relation between the two factors.

Except in the case of data for the milk from individual animals, all milk was standardized to a fat content of 3.5 per cent.

Pasteurization was carried out by the holding method. When the volume of milk warranted it, a 150-gallon "Jensen Cream Ripener" was used; otherwise the milk was placed in shotgun cans and immersed in a large vat of hot water. Heating was continued for thirty minutes after the milk had attained the desired pasteurizing temperature. Cooling was brought about as quickly as possible by means of a vat of cold water.

Cream layers were measured on samples of milk placed in 200 cc. flat bottom, graduated cylinders. Usually the milk was at a temperature of 26°C. when placed in the cylinders. Four cylinders were used for each sample, two of which were cooled to 0°C. and the other two to 9° to 10°C. These temperatures were maintained for twenty-four hours, after which the depth of the cream was measured with dividers. One-half of the average reading was recorded as the percentage of cream.

In a number of cases an estimate was made of the distribution of fat between the cream and skim milk layer. For this purpose cylinders were employed having a hole about 3 mm. in diameter bored in the side a few millimeters from the base. These were kept stoppered until the end of the creaming period when the stopper was removed and the skim milk drawn off sowly to within $\frac{1}{4}$ inch of the cream layer. The percentage of fat in this layer was determined directly, using the Babcock test. The approximate percentage of fat in the cream layer was calculated by the formula,

$$C^* = \frac{x - \dfrac{(100 - y)z}{100}}{z} \times 100$$

where C = percentage fat in cream layer
x = percentage fat in the milk
y = percentage cream
z = percentage fat in skim layer

Specific gravity was determined by the lactometer (United States Dairy Division type). Solids-not-fat were calculated from the tables of Shaw and Eckles (6).

Hydrogen ion concentrations were determined potentiometrically using the Bailey electrode.

Viscosities were measured by the MacMichael viscosimeter, using a disc bob and a "00" torsion wire. The cup was not rotated; instead, the disc was rotated somewhat as in the Doolittle (7) method, as follows. The disc was first turned to the left one revolution and then allowed to rotate back through the milk until a maximum deflection was reached. This reading, subtracted from the reading obtained in air alone was taken as the comparative viscosity of the milk. In order that the results could be calculated in terms of centipoise units the instrument was calibrated by means of sugar solutions and a reference curve constructed. Although the method employed for the viscosity work was arbitrary, the results could thus be expressed in units such that the data can be verified (or otherwise) by other workers

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* This formula does not give strictly accurate results because it ignores the specific gravity of the skim milk and cream.

using any instrument from which similar calculations can be made.

Results

a. Influence of breed. The influence of breed on the viscosity of milk was first determined on a composite twenty-four-hour sample from each of 37 cows, representing 2 Ayrshire, 5 Jersey, 11 Guernsey, and 19 Holsteins. The average data for the breeds are shown in table 1.

These data do not indicate any relation between the hydrogen ion concentration and viscosity of milk, but there is a relation shown between both the fat content and solids-not-fat and viscosity. Inasmuch as the viscosity range is accompanied by

TABLE 1

The composition, viscosity, and other property of milk from different breeds of cows

BREED	FAT	SOLIDS-NOT-FAT	SPECIFIC GRAVITY	pH	VISCOSITY		
					At once at 25°C.	At once at 0° C.	After twenty-four hours at 0° C.
	per cent	*per cent*			*cp.*	*cp.*	*cp.*
Jersey..........................	5.62	9.64	1.034	6.47	2.77	5.67	5.71
Guernsey......................	4.77	9.37	1.0336	6.44	2.37	5.18	5.44
Ayrshire.......................	4.62	9.13	1.0331	6.70	2.39	5.16	5.88
Holstein.......................	3.41	8.81	1.0325	6.44	1.90	4.57	4.63

a wider range of fat than of solids-not-fat, the data suggest that the latter exerts the greater effect. In the case of market milk, however, where creaming variations are of special importance, the percentage of fat has practically no bearing on these variations because of the almost universal practice of standardization. The relation of viscosity to creaming variations must accordingly be sought in the effects exerted by the size of fat globules and by the solids-not-fat.

The relation of fat-globule size, or more correctly the relative number of fat globules, to viscosity is illustrated best by the well-known effect of homogenization which increases the viscosity very greatly. While this is an extreme case not met in market milk,

it suggests that there may be sufficient breed difference with respect to the relative size of the fat globules to be a factor in creaming.

b. *Relative effect of size of fat globules and content of solids-not-fat on viscosity and creaming.* The opinion is held (8) that the milk from the Jersey and Guernsey breeds creams more easily than that of the Holsteins and Ayrshires because the larger fat globules have a greater mass in proportion to their surface. This hypothesis was tested by two experiments as follows. In the first experiment Holstein skim milk was standardized to 3.5

TABLE 2

Relative influence of solids-not-fat and size of fat globules on viscosity and creaming

EXPERI-MENT NUMBER	SOURCE OF PLASMA	SOURCE OF FAT GLOBULES	SOLIDS-NOT-FAT	PROPERTIES AT 0°C.		PROPERTIES AT 9° TO 10°C.	
				Viscosity after twenty-four hours	Volume of cream	Viscosity after twenty-four hours	Volume of cream
			per cent	*cp.*	*per cent*	*cp.*	*per cent*
1	Holstein	Holstein	8.59	5.32	11.30	2.89	6.80
	Holstein	Jersey-Guernsey	8.61	5.38	13.01	3.13	9.30
	Jersey-Guernsey	Jersey-Guernsey	9.35	6.05	17.40	3.36	12.80
	Jersey-Guernsey	Holstein	9.30	5.93	16.51	3.58	11.30
2	Holstein	Holstein	8.79	5.24	12.41	2.93	7.51
	Holstein	Jersey-Guernsey	9.11	5.87	11.81	3.01	6.41
	Jersey-Guernsey	Jersey-Guernsey	9.61	6.41	16.80	3.25	14.51
	Jersey-Guernsey	Holstein	9.44	6.19	16.71	3.77	10.91

per cent fat with freshly separated cream (55 per cent fat) from Jersey-Guernsey milk, and an analogous milk prepared from Jersey-Guernsey skim milk and Holstein cream. The properties of these milks were compared with those of unaltered Holstein and Jersey-Guernsey milk except as was necessary to bring the fat content to 3.5 per cent. In the second experiment all the milks were prepared from skim milk and cream. The results are given in table 2. They show clearly that with a uniform fat content such variation in the size of the fat globules as results from breed differences is relatively unimportant in determining

either viscosity or creaming. On the other hand, the data show a direct relation between the concentration of solids-not-fat and viscosity; the data also indicate a close relation between creaming and viscosity, but the relationship does not appear to be an absolute one. For example, in both experiments practically as good cream layers were obtained at 9° to 10°C. with the Jersey-Guernsey plasma as at 0° with Holstein plasma, although the viscosity in the former case averaged about 3.5 and in the latter about 5.4. A closer relationship between viscosity and creaming

TABLE 3

Viscosity, creaming, and fat distribution between skim milk and cream of raw and pasteurized milk of uniform composition

EXPERIMENT NUMBER	TEMPERATURE OF PASTEURIZATION	PROPERTIES AT 0°C.				PROPERTIES AT 12°C.			
		Viscosity after twenty-four hours	Cream volume	Concentration fat in skim	Approximate concentration fat in cream	Viscosity after twenty-four hours	Cream volume	Concentration fat in skim	Approximate concentration fat in cream
	°C.	cp.	per cent	per cent	per cent	cp.	per cent	per cent	per cent
1 {	Raw	5.45	14.8	0.34	21.7	3.12	11.6	1.12	21.6
	60	5.57	13.6	0.23	24.2	3.03	11.7	0.85	23.5
2 {	Raw	5.34	16.4	0.27	20.0	2.93	11.7	1.20	20.9
	62	4.80	14.9	0.35	21.5	2.89	10.8	0.90	25.0
3 {	Raw	4.73	15.4	1.00	17.2	3.10	10.5	1.50	20.6
	65	4.45	13.5	1.20	18.2	2.93	10.0	1.22	24.8
4 {	Raw	5.02	15.9	0.52	19.3	2.75	9.8	1.52	21.7
	67	4.28	11.1	1.30	21.1	2.87	9.0	1.42	24.5

is seen when comparing the cream volume and viscosity at 0°C. with the same data at 9° to 10°C. The percentage decline at the higher temperatures is not identical for both viscosity and cream volume, but is as uniform for each factor as could be expected. Calculation shows an average decrease in viscosity of 43.8 per cent accompanied by an average decrease of 32.5 per cent in cream volume.

    c. Viscosity change as a factor in the influence of pasteurization on creaming. The results obtained with raw milk suggested

that the detrimental effect of pasteurization on creaming might be explained, at least in part, by changes in viscosity.

Two groups of experiments were conducted, the first with mixed

TABLE 4

Viscosity, creaming, and fat distribution between skim milk and cream, of raw and pasteurized milk of uniform fat content and varying content of solids-not-fat and varying size of fat globules

EXPERIMENT NUMBER	TEMPERATURE OF PASTEURIZATION	SOLIDS-NOT-FAT*	FAT GLOBULES†	PROPERTIES AT 0°C.				PROPERTIES AT 12°C.			
				Viscosity after twenty-four hours	Cream volume	Concentration fat in skim	Approximate concentration fat in cream	Viscosity after twenty-four hours	Cream volume	Concentration fat in skim	Approximate concentration fat in cream
	°C.	per cent		cp.	per cent	per cent	per cent	cp.	per cent	per cent	per cent
1	Raw	8.71	Small	4.05	14.3	1.00	18.4	2.54	13.4	1.37	17.3
	62	8.71	Small	3.94	11.0	1.40	20.5	2.43	8.5	1.87	19.9
	Raw	9.47	Large	4.84	17.0	0.37	18.8	2.89	14.3	0.90	19.1
	63	9.47	Large	4.62	11.0	1.30	21.3	2.89	7.3	2.00	22.6
2	Raw	9.81	Large	4.22	16.4	0.90	16.8	2.63	7.0	1.97	23.8
	62	9.81	Large	4.10	11.4	1.20	21.3	2.59	6.4	1.87	27.3
	Raw	9.34	Small	4.28	14.3	1.00	14.5	2.66	5.1	2.45	23.1
	63	9.34	Small	4.28	2.0	3.10	22.4	2.47	1.5	2.97	38.3
3	Raw	9.19	Small	4.14	15.3	1.60	14.0	2.24	8.5	2.10	18.6
	63	9.19	Small	4.03	2.8	2.95	22.6	2.32	2.9	2.90	23.6
	Raw	8.99	Large	3.70	11.8	1.70	16.9	2.01	5.8	2.35	22.2
	63	8.99	Large	3.54	2.0	2.90	32.9	2.19	2.3	2.75	35.3
4	Raw	8.69	Small	4.22	10.8	2.50	11.8	2.32	7.1	2.55	15.9
	65	8.69	Small	3.94	1.5	3.30	16.7	2.19	1.4	3.05	35.1
	Raw	9.11	Large	4.10	6.4	2.70	15.2	2.43	6.0	2.60	17.6
	66	9.11	Large	4.45	1.5	3.30	16.7	2.89	1.5	3.20	23.2

* The lower solids-not-fat represents Holstein plasma, and the higher Jersey-Guernsey plasma.

† Small fat globules represent Holstein cream and large fat globules Jersey-Guernsey cream.

milk in vat lots and the second in smaller lots using milk in which the solids-not-fat and size of fat globules were varied as in part (*b*). The temperatures of pasteurization varied from 60° to

67°C. for the different lots of mixed milk and from 62° to 65.8°C. for the milk with varying content of solids-not-fat. In both experiments the fat content was adjusted to 3.5 per cent. The data for the first group of experiments are summarized in table 3, and for the second group of experiments in table 4. An average of all data collected on raw and pasteurized milk is given in table 5. In this average are included a number of other experiments not reported in this paper in which a study was made of the effect of various milk plant operations on cream rising.

When examining the properties of the milk at 0°C. as shown in tables 3, 4, and 5, it appears as though the decrease in cream volumes resulting from pasteurization is more or less related to a lowered viscosity. This apparent relation becomes much less

TABLE 5

Average relation found between viscosity, creaming, pasteurization and distribution of fat between skim milk and cream

NUMBER OF SAMPLES	TEMPERA-TURE OF CREAMING	TREATMENT	VISCOSITY AFTER TWENTY-FOUR HOURS	CREAM VOLUME	CONCEN-TRATION FAT IN SKIM	APPROXI-MATE CONCEN-TRATION FAT IN CREAM
	°C.		cp.	per cent	per cent	per cent
46	0	Raw	5.10	15.6	0.76	18.3
16	0	Pasteurized	4.47	9.1	1.58	22.7
46	9–12	Raw	3.01	10.6	1.45	20.8
16	9–12	Pasteurized	2.71	7.1	1.84	25.2

significant when examining the data for milks with varying content of solids-not-fat and having a different initial viscosity. The relationship practically disappears for the milk creamed at the higher temperature where many cases occurred showing no effect of pasteurization on viscosity and great effect on creaming. It is true that the average of all the data collected (table 5) indicates a general relationship between viscosity and creaming as affected by pasteurization. Calculation shows, however, that while there is no correspondence between the percentage decline in viscosity and the percentage loss in cream volume, there is a rather close proportion between the two when comparing the results at the two creaming temperatures. The ratio between viscosity decrease and cream volume loss was 1:29.5 at 0°C. and 1:31.5 at
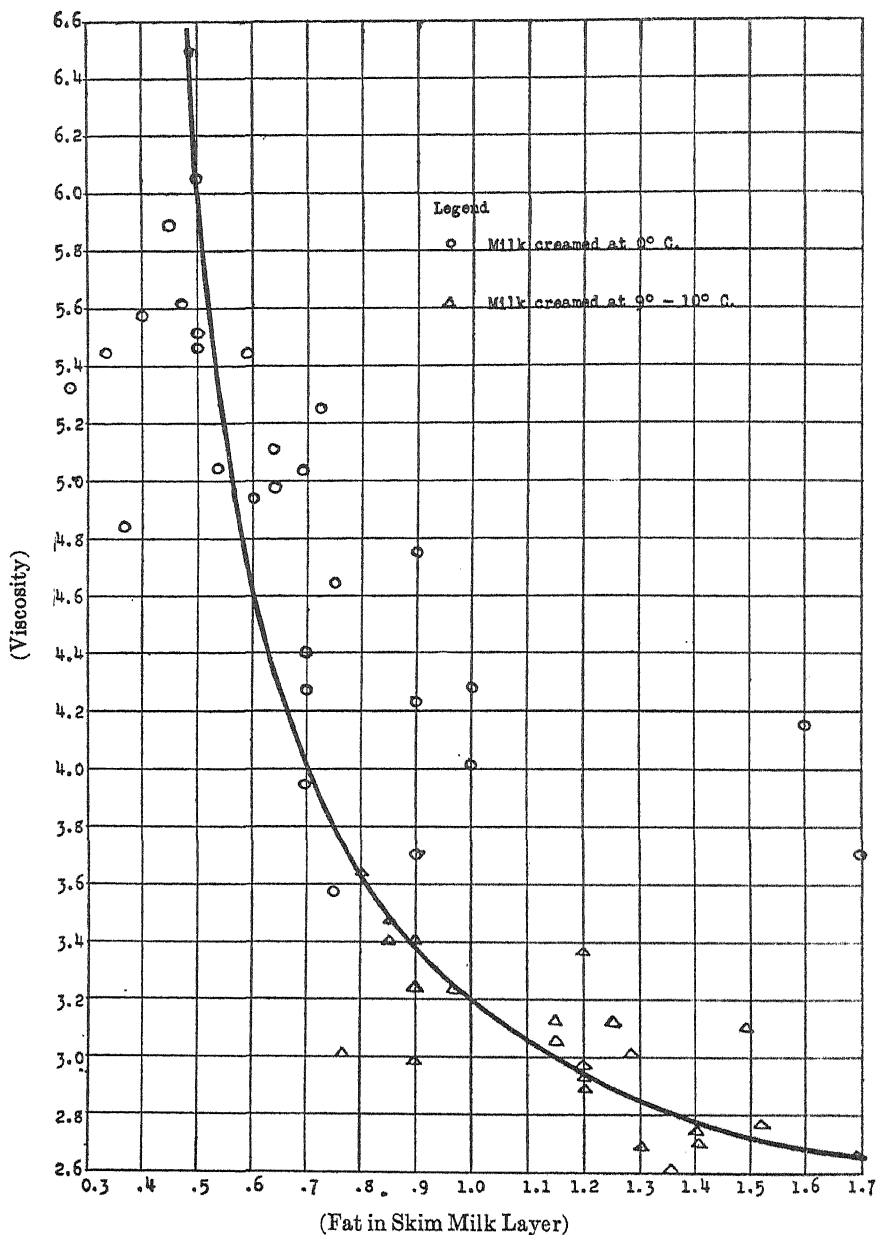
FIG. 1. RELATION BETWEEN VISCOSITY OF RAW WHOLE MILK AND THE PER-
CENTAGE OF FAT IN THE SKIM MILK LAYER AFTER CREAMING

9° to 12°C. However, these ratios are too wide to warrant the conclusion that viscosity is a major index of the detrimental effect of pasteurization on cream rising.

d. Influence of pasteurization on fat distribution in skim milk and cream layers. Much more significant are the data on the distribution of fat between the cream and skim milk layers. In a great majority of cases a loss in cream volume due to heating was accompanied by a higher percentage of fat in the skim milk, and in every instance by a higher percentage of fat in the cream. This shows clearly that losses in cream volume because of heating the milk are often due to less exhaustive creaming and particularly to a closer packing of the fat globules in the cream layer.

A complete explanation of the cause of the less exhaustive creaming awaits further study. Figure 1 shows that there is some relation between this result and the viscosity in the case of raw milk. The points on the chart are taken in part from the data in tables 3 and 4 and in part from other experiments not reported. While the points do not fall on the curve as closely as may be desired, the general relationship is shown very definitely. A similar correlation does not exist for pasteurized milk.

The causes underlying the closer packing in the cream layer are also little understood. Our results are similar to those obtained by Weinlig (9). He believes that the increased concentrations of fat in the cream layer which he observed after pasteurizing milk at temperatures above 63° to 64°C. are due to changes in the composition of the membrane material around the fat globules, giving rise to a decreased tenacity. The problem, however, requires further study.

DISCUSSION

The results of this study show clearly that the plasma colloids are a much more important factor in determining cream volumes than are the fat globules themselves when studying milk with a uniform fat content. Inasmuch as the specific viscosity of milk is due largely to the plasma colloids it is not surprising that the cream volume rising on raw milk is affected by such factors as influence the viscosity of the plasma. Certainly the difference

in viscosity of milk at 0°C. compared with 9° to 12°C. is sufficient to account for the variations in the cream layer at the two temperatures. However, the variations in the depth of the cream layer at the same creaming temperature are not accounted for wholly by differences in viscosity.

The detrimental effect of pasteurization on cream volumes is only explained in part by viscosity changes. The decrease in viscosity of pasteurized milk is too small to account for the decrease in depth of the cream layer when compared with raw milk. If viscosity were a true index of cream rising the great increase in viscosity accompanying a lowering of the creaming temperature would be expected to overcome the slight decrease in viscosity due to the heat. On the contrary the results show that while the viscosity of pasteurized milk is still relatively high at 0°C., in comparison with the viscosity at 9° to 12°C. the cream volume at lower temperatures are not proportionate to the higher viscosity at that temperature. It is therefore obvious that pasteurization effects are not explainable on the basis of viscosity.

The relation of the concentration of plasma solids to cream volumes on raw milk is shown very strikingly by the experiments on milk with a uniform fat content in which cream and plasma were interchanged to give different levels of solids-not-fat and a different relative size of the fat globules. When the solids-not-fat were higher a better cream layer resulted regardless of the relative size of the fat-globules. It is recognized that the data show some favorable influence due to larger fat globules, but it is not very great. It is clear, however, why the breed differences in cream layers on raw milk are so striking, especially when comparing Holstein with Jersey or Guernsey milk. In the former lower plasma solids are accompanied by both relatively small fat globules and lower fat content while in the latter higher plasma solids are associated with both larger globules and more fat. Pasteurization, however, tends to minimize these differences. For pasteurized milk just as poor cream layers are apparently obtained in milk with the higher plasma solids as with the lower.

The distribution of fat between the skim milk and cream layers

of pasteurized milk is very suggestive for a further study of the fundamental factors causing losses in cream volumes. It was pointed out in the introductory paragraphs that one of the two important general factors determining the depth of cream layers is the volume occupied by the risen fat globules. This factor is seen in a very striking manner in our experiments showing that there is a distinctly closer packing of the fat globules in the cream which rises on pasteurized milk. It is of interest to note, also, that the cream which formed in many of our experiments was richer in fat than the standard 18 per cent product distributed by many dairies as cream.

CONCLUSIONS

1. The volume of cream rising on raw milk of uniform fat content is determined largely by the content of solids-not-fat in the plasma.

2. The specific viscosity of raw milk is determined in large measure by the content of plasma solids and by the temperature at which the plasma is held.

3. The viscosity of raw milk is a good index of its creaming ability and can be used as an explanation of changes in cream layers caused by the temperature of creaming or the concentration of plasma solids in the milk.

4. Viscosity is only a minor factor in determining the cream layers on pasteurized milk; the fundamental factors having an adverse effect remain to be determined.

5. The deterimental effect of pasteurization on cream volumes is accompanied by (a) less exhaustive creaming and (b) closer packing of the fat globules in the cream layer. Neither of these effects are explainable by viscosity changes. In raw milk, however, there is a general relation between viscosity and exhaustiveness of creaming.

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THE MAINTENANCE REQUIREMENT OF CATTLE FOR PROTEIN, AS INDICATED BY THE FASTING KATABOLISM OF DRY COWS[*][1]

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The study here reported is based on experiments with fasting, dry cows, and was designed primarily to add to the knowledge of the energy metabolism of cattle. The collection and analysis of the excreta during fast, however, yielded data of significance in relation to protein requirements, and these constitute the basis of the present paper. The results of the heat measurements and of the gaseous exchange will be treated elsewhere.

OUTLINE OF THE INVESTIGATION

The schedule of experimental treatment will be found in table 1.

Four dry Jersey cows were used as subjects in this investigation and are designated nos. 874, 885, 886 and 887. These are the same animals as were used in the experiments on "The maintenance requirement of dry cows" which have been already reported (2). The fasting experiments with these animals were conducted during the same years as the maintenance experiments, the periods of fast following the periods in which the animals received maintenance rations in respect to energy. In experiment 221D, 1920, cows 885 and 886 were used, each being six years old. In experiment 221E, 1921, cow 885 was used. In experiment 221F, 1922, cow 874, six years old, and

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TABLE 1

Schedule of starvation and preceding maintenance periods

EXPERIMENT AND ANIMAL NUMBER	FEEDING PERIODS PRECEDING FAST						Duration of fast	STARVATION PERIODS		
	Dates	Daily ration				Daily gain of nitrogen by body		Date and time of offering last feed	Intervals of time represented by excreta collected	Dates of calorimeter measurements
		Hay.	Grain	Nitrogen						
				Total	Apparently digested					
		kgm.	*kgm.*	*gms.*	*gms.*	*gms.*	*days*			
221D—886	January 25 to March 22, 1920	1.745	2.614	95.6	71.5	+6.7	3	March 22, 5 p.m.	March 22, 8 a.m. to March 25, 6 p.m.	March 23 to 25
885	February 8 to April 5, 1920	1.779	2.668	97.3	75.7	+4.5	3	April 5, 5 p.m.	April 5, 8 a.m. to April 8, 6 p.m.	April 6 to 8
221E—885	February 12 to March 11, 1921	1.580	2.360	88.3	68.8	+4.5	6	March 11, 5 p.m.	March 11, 8 a.m. to March 17, 6 p.m.	March 15 to 17
221F—874	January 7 to February 1, 1922	1.830	2.744	97.4	73.1	+2.1	9	February 1, 6 a.m.	February 1, 8 a.m. to February 10, 8 a.m.	February 7 to 9
887	March 1, to March 22, 1922	1.650	2.474	89.0	68.5	−3.8	9	March 22, 6 a.m.	March 22, 8 a.m. to March 31, 8 a.m.	March 28 to 30

cow 887, five years old, were used. The length of the fasting periods was 3 days in experiment 221D, six days in experiment 221E, and nine days in experiment 221F. The animals were kept on approximately maintenance rations of alfalfa hay and a grain mixture for at least three weeks preceding the fasting experiments. The grain mixture consisted of 30 parts by weight of wheat bran, 30 parts of ground oats, 30 parts of corn meal and 10 parts of old process linseed meal.

During the fasting experiments, and the preceding maintenance periods, the animals were kept in the Institute barn, except during the last two days of each experimental period, which were passed in the calorimeter. The animals were offered water at 8:00 a.m. in the barn, and at 7:00 a.m., in the calorimeter.

Feces and urine were collected as a mixture, the daily collection being at 8:00 a.m., in the barn, and at 6:00 p.m., in the calorimeter. During the last few days of the fast, in experiments 221E and 221F, the feces were of such solid consistency that it was practicable, in some cases, to make an approximately complete separation of the urine from the feces by decanting, and straining the urine through cheesecloth. Such a separation was made of the excreta collected during thirty-four hours preceding the last day of the fast in experiment 221E, and during the last four days of the fast in experiment 221F. In the tables the data relating to these separated excreta are designated by the letters U and F following the weights of the fresh urine and feces.

The feces-and-urine mixtures and the separated excreta were subjected to the usual complete analysis, but only the dry matter, crude fiber and nitrogen will be here considered. Nitrogen was determined in the fresh feces, and feces-and-urine mixtures, by König's method, and in the urine by the Kjeldahl method.

THE EFFECT OF FASTING ON THE EXPERIMENTAL ANIMALS

The animals showed no physical ill-effects of the fasting. Apparently they lost the natural craving for feed at about the

third day. Close observation showed no unusual actions other than the failure to ruminate, and the rather frequent moistening of the muzzle with the tongue.

At the close of the fasting period, alfalfa hay was offered the animals. In each case after taking but a few mouthfuls the

TABLE 2

Daily record of live weight and of water drunk during fast

EXPERIMENT, ANIMAL, AND PERIOD	DATE	LIVE WEIGHT	WATER DRUNK	EXPERIMENT, ANIMAL, AND PERIOD	DATE	LIVE WEIGHT	WATER DRUNK
	1920	*kgm.*	*kgm.*		*1922*	*kgm.*	*kgm.*
221D				221F			
886-IV	March 22		11.0	874-III	February 1		12.8
	March 23	419.0*	0.0		February 2	417.2*	0.0
	March 23	416.9†	—		February 3	409.6*	0.0
	March 24		0.0		February 4	404.0*	7.2
	March 25	402.3‡	0.0		February 5	404.8*	0.0
					February 6	400.0*	4.0
885-IV	April 5		10.4		February 7	401.0*	0.0
					February 7	396.9†	—
	April 6	422.4*	0.8		February 8	—	8.3
	April 6	420.9†	—		February 9	398.7‡	1.2
	April 7		2.7		February 10	391.7*	0.0
	April 8	415.9‡	5.2				
	1921			887-III	March 22		15.4
221E					March 23	319.0*	0.0
885-III	March 11		8.4		March 24	312.4*	0.0
	March 12	429.8*	5.2		March 25	307.4*	4.6
	March 13	426.4*	5.9		March 26	305.3*	9.3
	March 14	428.4*	0.0		March 27	308.0*	3.0
	March 15	422.0*	3.2		March 28	304.6*	0.0
	March 15	424.2†	—		March 28	303.9†	—
	March 16		2.0		March 29	—	0.4
	March 17	417.6‡	4.4		March 30	294.9‡	7.4
					March 31	290.1*	0.0

* Before drinking, at 8:00 a.m.
† At 1:00 p.m.
‡ At 6:00 p.m.

animals lay down. After about an hour more hay was taken, and within twenty-four hours the animals were eating normally.

DAILY RECORD OF LIVE WEIGHTS AND WATER DRUNK

In table 2 the daily live weights of the animals, and the water drunk during the fast, have been collected for reference. The

animals could not be weighed while in the respiration calorimeter, but were weighed before entering and upon leaving the chamber. Attention is directed to the wide variation in the daily amounts of water drunk during the fast. With one exception (cow 885) the animals refused to drink on the first and second days of the fast.

THE DAILY EXCRETION OF FECES AND URINE BY THE FASTING COWS

Table 3 presents data relative to the daily excretion of feces and urine. These data exhibit great irregularity, and render obvious the difficulty of getting a true picture of the daily excretion of fasting cows. For example, in experiment 221F, cow 874 excreted neither urine nor feces during the twenty-four hours ending February 9, 6:00 p.m., while during the following fourteen hours (ending February 10, 8:00 a.m.) she excreted 7.9 kgm. of urine and feces, containing 81.2 grams of total nitrogen, of which 66.9 grams were contained in the urine. This amount of urinary nitrogen is greater than the amount of nitrogen excreted in both urine and feces during any one of the preceding six days, and, if computed per day, would be greater than that excreted in both urine and feces during the three preceding days combined. This demonstrates the futility of attempting to picture the daily decrease of nitrogen excretion from data secured by arbitrarily separating the excretion into twenty-four-hour periods, and shows the necessity, in accurate work, of catheterizing the subjects at the beginning and at the close of an experimental period.

Of especial interest is the persistance of crude fiber in the feces of cows 874 and 887 during the nine-day fasting periods. From this it is obvious that even during nine days of starvation the alimentary tract is not completely freed from feed residues, though the amounts of crude fiber eliminated during the latter part of the fasting periods are comparatively slight.

THE PROTEIN KATABOLISM OF FASTING

The nitrogen of the urine during short fasting periods must be considered to contain a small and rapidly diminishing quota

TABLE 3

The daily excretion of feces and urine by the fasting cows

EXPERIMENT, ANIMAL, AND PERIOD	DATE AND TIME OF ENDING DAILY COLLECTION	TIME COVERED BY COLLECTION	TIME INTERVAL BETWEEN LAST FEEDING AND END OF COLLECTION		WEIGHT OF FRESH EXCRETA	DRY MATTER OF EXCRETA*		CRUDE FIBER		NITROGEN		
								Dry matter basis	Weight	Fresh basis	Urine and feces separated	Urine and feces mixed
		hours	days	hours	kgm.	per cent	grams	per cent	grams	per cent	grams	grams
221D												
886–IV	*1920*											
	March 23, 8 a.m.	24	0	15	11.124	12.75	1,418.1	29.49	418.2	1.02		113.0
	March 23, 6 p.m.	10	1	1	4.863	11.97	581.9	31.61	183.9	0.76		36.7
	March 24, 6 p.m.	24	2	1	4.460	10.38	463.0	27.71	128.3	1.46		65.2
	March 25, 6 p.m.	24	3	1	2.823	16.17	456.5	34.79	158.8	1.28		36.2
885–IV	*1921*											
	April 6, 8 a.m.	24	0	15	12.274	11.77	1,444.0	31.57	455.8	0.83		102.2
	April 6, 6 p.m.	10	1	1	5.968	13.40	799.9	35.62	284.9	0.49		29.3
	April 7, 6 p.m.	24	2	1	3.252	8.89	289.2	19.37	56.0	1.98		64.3
	April 8, 6 p.m.	24	3	1	0.826†	21.49	177.5	34.49	61.2	0.63		5.2
221E												
885–III												
	March 12, 8 a.m.	24	0	15	9.150	12.97	1,186.3	32.19	381.9	0.86		78.2
	March 13, 8 a.m.	24	1	15	4.416	9.07	400.7	26.19	104.9	1.51		66.7
	March 14, 8 a.m.	24	2	15	1.200	22.56	270.7	36.90	99.9	0.49		5.9
	March 15, 8 a.m.	24	3	15	4.470	12.11	541.5	29.82	161.5	1.53		68.3
	March 15, 6 p.m.	10	4	1	{1.518 U. / 0.240 F.}	— / 10.64	— / 25.5	— / 29.90	— / 7.6	1.85 / 1.83	28.0 / 4.4	32.4
	March 16, 6 p.m.	24	5	1	{1.804 U. / 0.126 F.}	— / 12.66	— / 16.0	— / —‡	— / —‡	1.47 / 1.55	26.5 / 2.0	28.5
	March 17, 6 p.m.	24	6	1	4.790	9.00	431.0	27.69	119.4	1.19		56.8
221F												
874–III	*1922*											
	February 2, 8 a.m.	24	1	2	7.860	17.15	1,348.0	25.61	345.2	1.03		85.0
	February 3, 8 a.m.	24	2	2	5.160	14.28	737.0	29.23	215.4	1.46		75.4

Date											
February 4, 8 a.m.	24	3	2	3.800	14.71	559.0	31.24	174.6	1.20		45.6
February 5, 8 a.m.	24	4	2	3.110§	4.67	145.1	—	—	1.01		31.3
February 6, 8 a.m.	24	5	2	3.000	11.20	336.0	32.22	108.3	0.97		29.1
February 7, 8 a.m.	24	6	2	1.978 U.	5.59	110.6	—	—	1.43	28.2 ⎫	38.9
				0.902 F.	19.60	177.0	33.39	59.1	1.19	10.7 ⎭	
February 7, 6 p.m.	10	6	12	1.670 U.	6.41	107.1	—	—	1.49	24.9 ⎫	29.4
				0.490 F.	22.29	109.0	34.64	37.8	0.92	4.5 ⎭	
February 8, 6 p.m.	24	7	12	2.700 U.	6.23	168.1	—	—	1.59	42.8 ⎫	45.0
				0.260 F.	21.89	57.0	35.70	20.3	0.84	2.2 ⎭	
February 9, 6 p.m.	24	8	12	0.000	—	0.0	—	—	—	0.0	00.0
February 10, 8 a.m.	14	9	2	6.300 U.	4.02	253.0	—	—	1.06	66.9 ⎫	81.2
				1.630 F.	18.67	304.0	37.60	114.3	0.88	14.3 ⎭	
887–III											
March 23, 8 a.m.	24	1	2	13.830	10.85	1,500.0	26.72	400.8	0.83		114.7
March 24, 8 a.m.	24	2	2	4.720	11.43	539.0	28.70	154.7	1.43		67.4
March 25, 8 a.m.	24	3	2	1.860	15.59	290.0	33.46	97.0	1.25		23.3
March 26, 8 a.m.	24	4	2	5.240	10.45	548.0	28.37	155.5	1.47		77.0
March 27, 8 a.m.	24	5	2	3.570	8.54	305.0	28.47	86.8	1.33		47.6
March 28, 8 a.m.	24	6	2	2.112 U.	2.49	52.6	—	—	1.00	21.1 ⎫	40.7
				2.118 F.	13.18	279.0	38.66	107.9	0.93	19.6 ⎭	
March 28, 6 p.m.	10	6	12	1.124†	10.01	113.0	39.77	44.9	0.91	10.3	10.3
March 29, 6 p.m.	24	7	12	6.088 U.	2.52	153.4	—	—	0.66	40.1 ⎫	43.7
				0.482 F.	14.42	69.0	41.34	28.5	0.74	3.6 ⎭	
March 30, 6 p.m.	24	8	12	3.462 U.	3.52	121.8	—	—	0.93	32.0 ⎫	44.1
				1.564 F.	15.80	247.0	41.95	103.6	0.77	12.1 ⎭	
March 31, 8 a.m.	14	9	2	3.645 U.	1.62	59.1	—	—	0.59	21.5 ⎫	24.4
				0.480 F.	13.56	65.0	42.20	27.4	0.60	2.9 ⎭	

Note: U. denotes urine; F. denotes feces.

* As determined, uncorrected for loss on drying.

† All feces.

‡ Disregarded; quantity negligible.

§ All urine.

TABLE 4

Average daily excretion of feces and urine by the fasting cows (based on data of table 3)

EXPERIMENT, ANIMAL, PERIOD	DURATION OF FAST	COLLECTIONS OF EXCRETA UPON WHICH AVERAGE PER DAY IS BASED	FRESH WEIGHT	DRY MATTER	CRUDE FIBER	NITROGEN In feces and urine	In urine
	days		*grams*	*grams*	*grams*	*grams*	
221D							
886-IV	3	Average, all	6.810	854.5	260.3	73.5	
		Average, omitting collection of first 24 hours	5.026	621.3	194.9	57.1	
		Average, last two days' collections	3.642	459.8	143.6	50.7	
885-IV	3	Average, all	6.533	793.3	251.1	58.8	
		Average, omitting collection of first 24 hours	4.157	524.1	166.4	40.9	
		Average, last two days' collections	2.039	233.4	58.6	34.8	
221E							
885-III	6	Average, all	4.319		136.4	52.5	
		Average, omitting collection of first day	3.427		103.7	47.7	
		Average, omitting collection of first two days	3.203		103.4	43.5	
		Average, omitting collection of first three days	3.790		104.5	54.4	
		Average, omitting collection of first four days	3.508		52.6	48.7	
		Average, last two days' collections	3.360		59.7	42.7	
221F							
874-III	9	Average, all	4.318	490.1	119.4	51.2	
		Average, omitting collection of first day	3.875	382.9	91.1	47.0	
		Average, omitting collection of first two days	3.691	332.3	73.5	42.9	
		Average, omitting collection of first three days	3.673	294.5	56.6	42.5	
		Average, omitting collection of first four days	3.786	324.4	46.3	44.7	
		Average, omitting collection of first five days	3.983	321.5	43.1	48.6	40.7
		Average, omitting collection of first six days	4.350	332.7	44.7	51.9	44.9

887-III	9	Average, all	5.588	482.4	134.1	54.8	
		Average, omitting collection of first day	4.558	355.2	100.8	47.3	
		Average, omitting collection of first two days	4.535	329.0	93.1	44.4	
		Average, omitting collection of first three days	4.981	335.5	92.4	48.0	
		Average, omitting collection of first four days	4.929	293.0	79.8	42.2	
		Average, omitting collection of first five days	5.269	290.0	78.1	40.8	28.7
		Average, omitting collection of first six days	5.615	276.1	68.1	40.8	31.2

derived from feed residues remaining in the alimentary tract at the beginning of the fast. Because of the great irregularity of excretion of feces and urine, aside from other considerations, the data of table 3 do not show clearly the progress of the fast, and it is therefore difficult to determine how many days following the withholding of feed should be regarded as representing transition from feed to actual fasting, and whether or not the urinary nitrogen of the last four days of the nine-day fast may be regarded as representing exclusively katabolism of body protein. In order to reduce the effect of irregularity of excretion, and to facilitate the interpretation of the data, we have computed from the data of table 3 several averages of the daily excretions, first, basing the average on all collections during the fasting periods, and then omitting successively the collection of the first day, first two days, etc. These averages are given in table 4.

The data of table 4 should be studied in conjunction with the data of table 3 from which they were derived. In view of the fact that we have no available data for the urinary nitrogen of the three-day fasts and of the first few days of the longer fasting periods, it is impossible to state definitely when actual fasting, or the post-resorptive state, was reached. However, the great drop in excretion of total nitrogen between the first and third days in all experiments (table 3), and the practical uniformity of the average excretion of dry matter, crude fiber and total nitrogen, omitting successively the collection of the first two, three, four, five and six days of the nine-day fasting period (table 4), indicate very strongly that the excretion of the first two days after feeding cannot be regarded as representing fasting conditions, but that these conditions were reached soon thereafter, and that the urinary nitrogen of the last four days of the nine-day fasting periods may be safely regarded as not being affected to an important degree by the residual feed in the alimentary tract.

It will be observed (table 4) that the average daily excretion of nitrogen in feces and urine for the last three days of the nine-day fast, is greater, with cow 874, than any of the other averages.

This is evidently due to the extraordinarily heavy excretion during the last fourteen hours of the fast (table 3), which is included in the average, and to the smallness of the number of days upon which the average is based.

The average daily excretion of urinary nitrogen for the last four days, by cow 874, is 40.7 grams, and for the last three days is 44.9 grams. With cow 887 the average for the last four days is 28.7 grams, and the average for the last three days is 31.2 grams. These differences between the averages for the last four and the three last days of the nine-day fasting periods are also obviously due to the irregularity of the daily excretions; and on this account the average based on the larger number of days is considered more nearly to represent the protein katabolism of fasting.

In the periods of maintenance feeding which immediately preceded these fasting studies nitrogen equilibrium was approximated (see table 1) in the presence of slight gains of energy, with the ingestion of 97.4 grams of nitrogen by cow 874, of which 73.1 grams were apparently digested, and 89.0 grams of nitrogen by cow 887, of which 68.5 grams were apparently digested. The comparison of these values for digested nitrogen with the nitrogen excreted per day in the urine (40.7 and 28.7 grams) by these animals during fasting suggests that in the feeding periods they received a considerable surplus of protein above that required for maintenance, although this is not shown by the balances of nitrogen ($+2.1$ and -3.8 grams), from which we may infer that practically all the surplus protein was used by the animals for energy production, in which case, of course, it would be without effect on the nitrogen balance.

The live weights of cows 874 and 887 in these fasting experiments differed by almost 100 kgm. The average of the last four weighings (see table 2) is 397.1 kgm. for cow 874, and 298.4 kgm. for cow 887. On the basis of these averages and the figures for average daily excretion of urinary nitrogen, per head, during the last four days of the nine-day fasting periods, namely 40.7 grams for cow 874, and 28.7 grams for cow 887, the urinary nitrogen excretion of fasting, computed per 1000 pounds live

weight, per day, is 46.5 grams for cow 874, and 43.6 grams for cow 887. These nitrogen values, considered as representing protein katabolism of fasting, that is, katabolism of body protein, are equivalent (using the factor 6.0) to 279 grams, or 0.62 pounds, of protein for cow 874, and 261.6 grams, or 0.58 pounds, for cow 887. The average for the two cows is 270.3 grams, or 0.60 pound, of protein.

It is of significance to note that the protein katabolism of fasting of these animals is exactly the same as Armsby's (1) estimate of the maintenance requirement of digestible crude protein, namely, 0.6 pound per 1000 pounds live weight.

SUMMARY

Results are reported of the daily excretion of urine and feces, and their content of dry matter, crude fiber and nitrogen, from four dry, fasting cows, one fasting for a period of three days, one for periods of three and six days, and two others for a period of nine days.

The results indicate that the bulk of the residual feed which is contained in the intestinal tract at the beginning of a fast is eliminated during the first two or three days after feeding, but that, in the absence of treatment directed toward the freeing of the alimentary tract from excreta, as in these experiments, the elimination of the same is incomplete even after nine days of fasting.

The average daily nitrogen excreted in the urine during the last four days of the nine-day fasting periods, which is considered to represent, with fair accuracy, the protein katabolism of fasting, was 46.5 grams for one cow, and 43.6 grams for the other, per 1000 pounds live weight. These nitrogen values are equivalent to 0.62 pound, and 0.58 pound, of body protein, respectively, or 0.6 pound, as an average, per 1000 pounds live weight, which figure is identical with Armsby's published estimate (1) of the digestible crude protein maintenance requirement of cattle, and is 0.1 pound less than as in Morrison's standard (3) (0.7 pound), which may be considered as providing more liberally for reproduction and other exigencies of practice.

It should be borne in mind, however, that the above values for protein katabolized during fasting may not represent the minimum protein requirement during feeding, in which case an abundant supply of non-nitrogenous nutriment may reduce the katabolism of protein to an amount less than that prevailing during fast. These figures for protein katabolism of fasting, therefore, when used as measures of the protein maintenance requirement during feeding, must be considered as providing a certain margin of safety.

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SOME FACTORS AFFECTING THE GROWTH OF CERTAIN STRAINS OF P. ROQUEFORTI

I. BLUE MOLD[*][1]

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INTRODUCTION

Previous work on the manufacture and ripening of Wensleydale cheese (3) has shown that in certain cases good mold growth was developed in the cheese. In the same experiments other cheese using similar milk under identical conditions the blue mold failed to develop. As all cheese had been inoculated, it is inconceivable that an absence of mold spores can be responsible for the lack of mold growth in the cases specified. But rather, that the causes of lack of mold growth might be looked for, in some inhibiting factor, or absence of suitable conditions for growth.

The lack of oxygen in the cheese, as shown by Thom and Currie (10), can hardly be considered the only factor as the mold in the best cheese usually develops first in the center (8).

In the previous work on the process of manufacture of Wensleydale cheese (3) it is shown that a starter was added to the milk and that acidity was developed in the making process. It is shown that this acidity is due to the organisms added in the starter and to the organisms in the raw milk.

The work of Hammer and others (4) (5) shows clearly that marked differences exist in starters as regards the associate organisms they contain and the acids that are produced. That the type of acid formed by these organisms might play an important part in providing a suitable medium for the growth of the

mold has suggested itself.   Matheson (6) reporting on the manufacture of Roquefort cheese recommends a liberal use of starter but gives no details as to the type of starter to be employed. Benson states "the ordinary commercial starter of Bacterium lactis is, however, not the right thing," for Stilton or Wensleydale cheese (1).   It is conceivable that the difference of opinion might well exist owing to different types of starter having been used.

Arising from the foregoing considerations, the work reported in this paper has been pursued.

OBJECT OF WORK

The object has been to determine the influence on different strains of P. roqueforti of acetic and citric respectively using such percentage of the same as possibly might be found in sour milk (4) (5) (9).

CULTURES USED

The cultures of P. roqueforti used were:

No. 1, P. roqueforti secured from Dairy Division, Washington (2).

No. 16, Isolated from Wensleydale cheese (2).

No. 32, Isolated from Wensleydale cheese inoculated with culture 16 (3).

No. 33, Isolated from Wensleydale cheese inoculated with culture 16 (3).

MEDIA

Milk.   Sweet skim milk in which no bacterial change had taken place was used in every case.   Test tubes of uniform diameter were selected, filled with exactly 10 cc. of milk, plugged and sterilized.

Milk + citric acid.   A citric acid solution was made of such strength that it contained 0.0327 gram of citric acid ($C_6H_8O_7 + H_2O$) per cubic centimeter.   This was added to test tubes plugged and sterilized.   The milk and acid solution were well cooled and two lots of acid milk of high and low concentration were prepared by adding 1 cc. and 0.5 cc. respectively to each 10 cc. tube of milk, under sterile conditions.   The concentrations of citric

acid were, therefore, 0.272 and 0.1423 per cent ($C_6H_8O_7$, H_2O), respectively.

Milk + acetic acid. An acetic acid solution was made up of such strength that it contained 0.01008 gram of acetic acid per cubic centimeter. Owing to the volatile nature of acetic acid, 1 cc. of glacial acetic acid, assumed to be sterile, was added to 100 cc. of sterile water. The milk and acid solutions were thoroughly cooled and two lots of acid milk of high and low concentrations were prepared by adding 1.5 and 0.5 cc. respectively, to each 10 cc. tube of milk under sterile conditions. The concentrations of acetic acid in the milk were 0.131 and 0.048 per cent (CH_3COOH), respectively.

Standard medium. Standard medium originating in part from Czapeks formula (10) was made up as follows:

Distilled water	2000 cc.
Magnesium sulphate	1 gram
Dipotassium phosphate	2 grams
Potassium chloride	1 gram
S. ferrous sulphate	0.02 gram
Peptone	20 grams
Lactose	50 grams
Agar	30 grams

Standard medium + citric acid. Two concentrations of standard medium and citric acid were prepared of such strength that either 1 or 0.5 cc. of the solution of citric acid (0.0327 gram per cubic centimeter) were added to each 10 cc. of the standard medium; the media were then tubed and sterilized. By this method concentrations of citric acid were obtained of 0.272 and 0.1423 per cent ($C_6H_8O_7$, H_2O), respectively.

Standard medium + acetic acid. The acetic acid solution was prepared as in the case of the milk and acetic acid previously referred to. To each 10 cc. test tube of the sterile standard medium either 1.5 or 0.5 cc. of this solution (0.01008 gram CH_3COOH per cubic centimeter) were added under sterile conditions. By this method concentrations of acetic acid were obtained of 0.131 and 0.048 per cent CH_3COOH, respectively.

Inoculation.   The test tubes of milk were inoculated from stock cultures grown on potato agar (11) using a platinum needle.

The plate cultures were first poured and allowed to cool on a flat surface.   The inoculation was done from the cultures using a platinum needle, and every endeavor was made to develop but one colony and that in the middle of the plate.

Incubation.   All cultures were grown in a room temperature incubator which averaged 21°C. and maintained a temperature between 22.5° and 20°C.

Measurement of growth in milk.   The method used to determine the degree of growth in the milk, and modification of milk, was to hold the cultures ten days after inoculation and then determine the percentage of casein not digested.   Standard Methods of Analysis were used (7) and the Kjeldahl determinations were made by the Gunning method.

Measurement of growth on solid media.   The growth on the plates of solid media was recorded after the third day and then daily.   This record included:

> Margin, average width in millimeter
> Size, average diameter of colony
> Color of spores, when spores were formed
> Color of reverse of colony

In measuring the rate of growth, it was found that the average diameter of the colony in millimeters was the most satisfactory way of expressing the rate of growth of the mold.

DIFFERENT STRAINS OF P. ROQUEFORTI GROWN IN MILK AND ACIDIFIED MILK

Four varieties of milk media, as previously described, were used in this experiment (see table 1).   The high concentration of citric acid was not analyzed as considerable coagulation and precipitation of the casein took place when the acid was added.   Quantitative determinations were made of the degree to which the cultures of different strains of P. roqueforti digested the casein of these milks.

The conclusions to be drawn from table 1 are:

1. The mold cultures of P. roqueforti 1 and 33 respectively (Roquefort origin) have a greater power to digest the casein than have cultures 16 and 32 (Wensleydale origin); which findings confirm previous work (2) (3).

2. There is a tendency for low concentrations of citric and acetic acids to effect the digestion of casein in milk by P. roqueforti:

TABLE 1

Different strains of P. roqueforti grown in milk and acidified milk cultures grown at room temperature in test tubes for ten days

CULTURES	MILK		MILK + 0.5 cc. ACETIC ACID OR 0.048 PER CENT ACETIC ACID		MILK + 1.5 cc. ACETIC ACID OR 0.131 PER CENT ACETIC ACID		MILK + 0.5 cc. CITRIC ACID OR 0.1423 PER CENT CITRIC ACID	
	Per cent casein	Per cent of casein digested*	Per cent casein	Per cent of casein digested*	Per cent casein	Per cent of casein digested*	Per cent casein	Per cent of casein digested*
Uninoculated control.........	3.10		3.04		3.08		3.03	
1	2.20	29.0	2.35	22.7	2.51	18.5	2.18	28.1
33	2.08	32.9	2.28	25.0	2.18	29.2	1.87	38.3
Average........		30.9		23.8		23.8		33.2
16	2.79	10.0	2.96	2.6	2.83	8.1	2.68	11.6
32	2.87	7.4	2.91	4.3	2.82	8.4	2.67	11.9
Average........		8.7		3.4		8.2		11.7

* This figure represents the percentage of the total casein that has been rendered soluble.

a. Acetic acid tends to reduce this digestion of casein particularly in weak concentrations.

b. Citric acid tends to increase this digestion of casein.

DIFFERENT STRAINS OF P. ROQUEFORTI GROWN ON THE STANDARD MEDIUM AND ACIDIFIED STANDARD MEDIA

In this experiment five modifications of media were used—the standard medium, a high and low concentration of the standard media and citric acid (figs. 1 and 2).

Fig. 1

At room temperature

FIG. 2
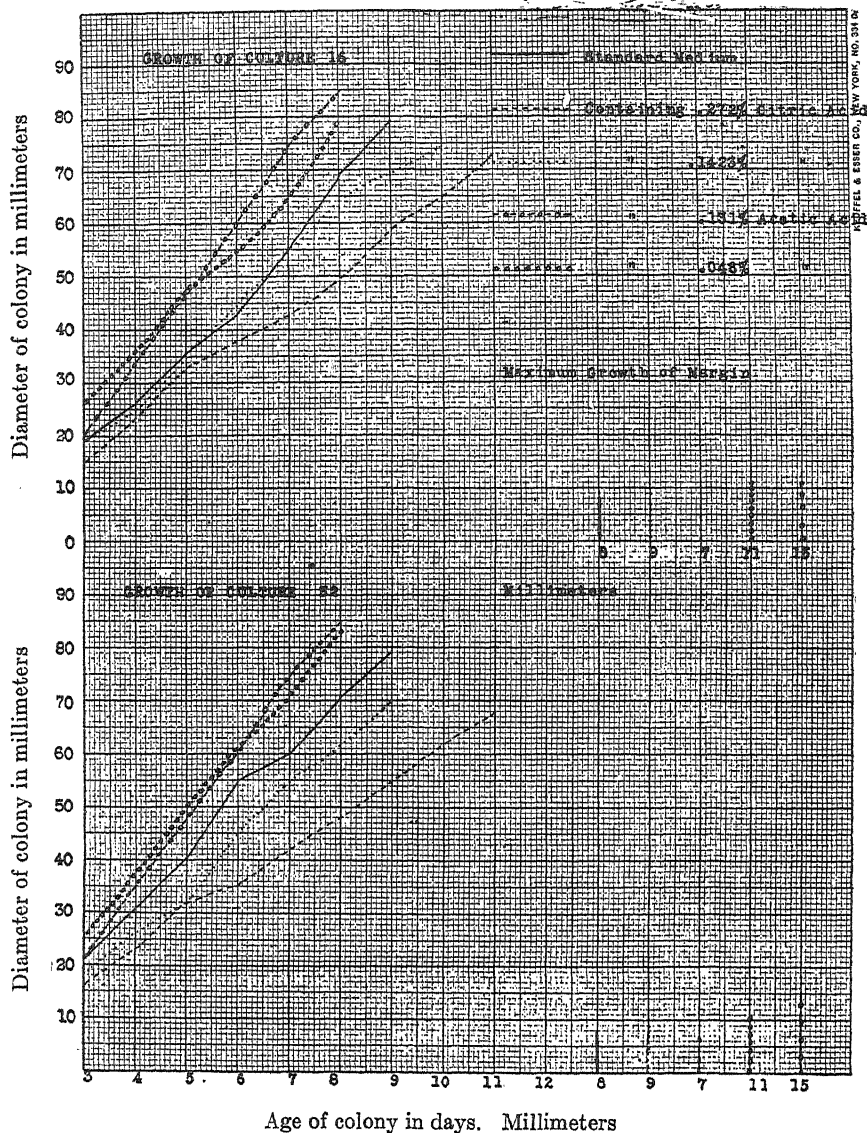
All plates were inoculated in duplicate and the average diameter of the colony on the two plates was measured daily and expressed in millimeters. The rate of growth has been expressed graphically for each culture, figures 1 and 2. The conclusions to be drawn from figures 1 and 2, are:

1. Citric and acetic acids when in small quantities in the standard media have a marked effect on the rate of growth of the mold.

2. Cultures 16, 32, 33, grow much faster where acetic acid is supplied to the standard media and this growth increases with the higher concentration.

3. Cultures 16, 32, 33, grow much slower where citric acid is supplied to the standard media and this growth decreases with the higher concentration.

4. Culture 1 does not follow this rule. This strain increases in growth with the presence of either acid, but shows a slight preference for acetic acid.

5. The width of the margin is in conformity with the rate of growth; the wider the margin the faster the growth.

From the other data recorded, the following facts are of significance:

1. In the case of cultures 16, 32, 33, the reverse of the colony is almost white, in plates containing high citric acid concentration; this effect is noticed to a less extent with the low concentration. In the case of culture 1, this effect was not observed.

2. Cultures 16 and 32, produce fewer spores and a quite distinctive hairy growth when grown on the media containing acetic acid.

SUMMARY

Citric acid and acetic acid in amounts comparable with those found in starters (4) (5) (9) have an effect on the growth of different strains of P. roqueforti, both as effecting the digestion of casein and growth on the standard media described. Low concentrations of acetic acid tend to reduce the digestion of casein in milk by strains of P. roqueforti while citric acid tends to increase this digestion.

On the other hand in the standard media acetic acid increases the growth while citric acid tends to inhibit it.

This work would indicate that the type of starter used in the manufacture of blue veined cheese might have a significant bearing on the subsequent growth of the mold in the cheese. At this stage it is advisable to point out that before definite conclusions can be drawn more work will have to be done, both culturally and as applied to the manufacture of blue veined cheese.

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THE RÔLE OF THE ANTISCORBUTIC VITAMIN IN THE NUTRITION OF CALVES*

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It is definitely known that man, the guinea pig and the monkey require a certain amount of the substance which is termed vitamin C or the antiscorbutic vitamin; and that unless the required amount of this vitamin is supplied by the food a hemorrhagic disease known as scurvy develops. It is also known that the rat, when fed on rations which produce scurvy in man, or in the animals mentioned, fails to develop the disease. The experimental work of Parsons and co-workers (1) (2) (3) and of Lepkovsky and Nelson (4) gives considerable evidence that the rat is able to synthesize the antiscorbutic vitamin and store it in the liver. These investigators consider this an indication that this rodent requires vitamin C and that its body is able to produce the vitamin for use even though there is little or none present in the feed.

A review of the literature on the subject of vitamin C does not reveal any instance on record where an experimental study has been made of the requirement of the bovine for the antiscorbutic factor. The need for definite knowledge regarding the role of the antiscorbutic vitamin in the nutrition of dairy cattle has been felt for some time both by veterinarians and by men interested in solving various problems in the field of cattle nutrition. Two outbreaks of a scurvy-like disease of cattle have

been reported, one from England, the other Australia, apparently caused by faulty nutrition. A striking similarity is noticeable between the characteristics of the disease as reported for these cattle and the well known symptoms of scurvy as found in guinea pigs which have been fed rations deficient in the antiscorbutic vitamin.

Place (5) of Australia says,

Look for a moment at a case of scurvy with its anemia and cachexia, its local hemorrhages and hemorrhagic inflammation, the spots upon the ribs, their dislocation from cartilages with perhaps neuritis and anesthesia of the limbs, and compare it with a coasty heifer with her bloodless eyes and bottled jaw, her gaspy breath, due to her ribs failing to distend, her tottering walk and swollen fetlocks, and finally her feeble, helpless efforts to control her limbs and raise her wasted carcass.

Place further states that "one of the symptoms of this form of disease in cattle (dental trouble) is a blue hyperemia of the gums and in some instances ulceration."

The occurrence of such a disease in England was noted by Little (6) who reports finding heifers, which had been fed on a ration that would undoubtedly produce scurvy in guinea pigs, showing many of the symptoms commonly noted in animals known to have scurvy. When first noticed some of these animals were dead or dying. On investigation Little diagnosed the cases as scurvy and was actually able to prevent further deaths by feeding either green grass, kohl rabi or swedes. Whether these outbreaks were due to a deficiency or absence of the antiscorbutic vitamin, or whether they were due to some other nutritional cause has been the point for considerable debate. The authors believe that the results of the experiment herein reported will throw light upon this particular question.

<div align="center">EXPERIMENTAL</div>

The first consideration in an experiment of this nature was to secure a ration which while deficient or lacking in vitamin C, would furnish adequate quantities of all the other nutritional factors necessary for life and growth. Since the requirement

of the bovine for many of these factors was at the time unknown it was necessary to base all considerations on the nutritional requirements of small laboratory animals.

The work reported in this paper was divided into two experiments. The first experiment gave significant results, but it was clear that the procedure could be improved considerably, and for this reason certain changes were made in the experimental methods followed in the second experiment.

Experiment 1

Ration. Specially treated alfalfa hay was the roughage fed during the first five months of experimental feeding. Treatment of this alfalfa consisted of autoclaving it for thirty minutes at a pressure of 15 pounds. Oat straw was substituted for the treated alfalfa hay during the fifth month because the demands of the calves for increased amounts of roughage became so great that it was difficult to autoclave as much alfalfa as the calves required.

Milk was fed to all the calves until they reached the age of six months. After considerable experimentation a definite method for treating the milk was adopted. The milk was heated to a temperature of 180°F. and held at that point for one hour. Throughout the time of holding oxygen from a storage drum was allowed to bubble through the milk at the rate of about 1 cubic foot per minute.

A grain mixture was fed consisting of 3 parts corn, 3 parts oats and 1 part old process linseed oil meal. To this mixture was added 5 per cent butterfat as a source of vitamin A and the antirachitic factor. One per cent of salt was also added. When the roughage was changed from alfalfa to timothy hay 183 grams of calcium carbonate were added to every 100 pounds of grain.

Animals. Four grade calves of uniform weight and appearance were used in this experiment. Two of these calves, E-3 and E-4, were used as experimental animals and received the ration deficient in the antiscorbutic factor. The other two, E-1 and E-2 were used as controls. The controls received the

same ration as the experimental calves with the exception that vitamin C was supplied. Lemon juice was first used as a source of this vitamin but the labor and expense involved soon caused
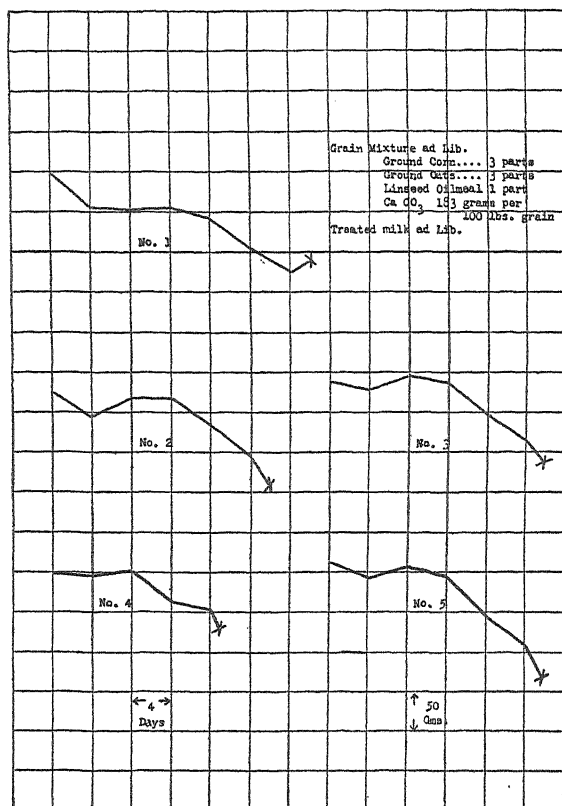
FIG. 1. WEIGHT CURVES OF GUINEA PIGS USED IN A FEEDING TRIAL TO DETER-
MINE THE SCORBUTIC POTENCY OF MILK WHICH HAS BEEN HELD FOR ONE
HOUR AT A TEMPERATURE OF 180° F. WHILE OXYGEN BUBBLED THROUGH IT
AT THE RATE OF ABOUT 1 CUBIC FOOT PER MINUTE

Horizontal squares represent four days, while vertical squares represent 50 grams.

the substitution of tomato juice and tomatoes were then used throughout the experiment.

During the early part of the experiment all of the feeds used were tested for their content of the antiscorbutic factor by feeding

trials with guinea pigs. All of the feed-stuffs used were shown to be deficient in vitamin C as they caused a rapid decline in the weight of the guinea pigs and finally death. Autopsy invariably showed very noticeable signs of scurvy. The data on one of the experiments, the results of which are typical of all the others, are presented in figure 1.

Effect of deficiency of vitamin C on growth and appearance of calves. Experimental feeding of the calves used in this trial
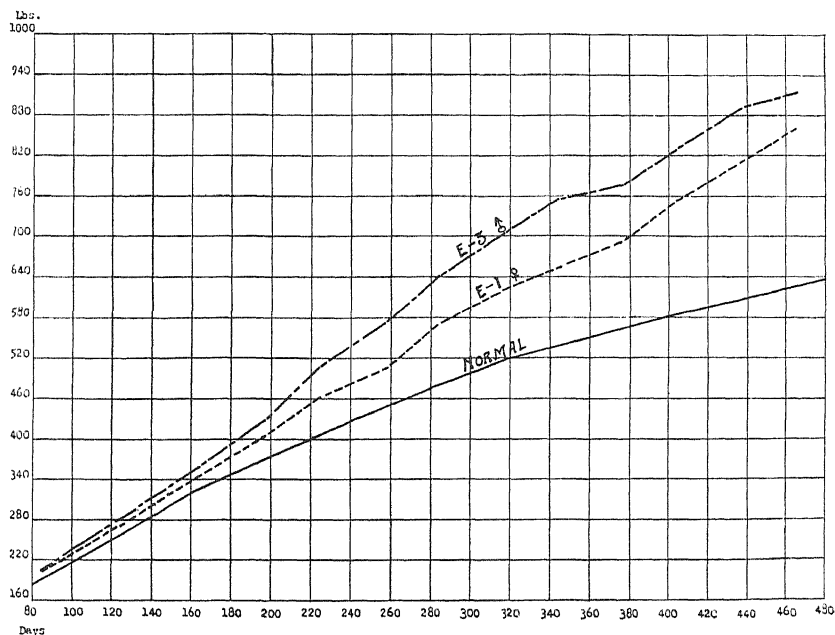
FIG. 2. GROWTH CURVES OF TWO OF THE CALVES USED IN EXPERIMENT 1

E-3 received a vitamin C deficient ration while E-1 received the same ration supplemented by tomato juice as a source of vitamin C.

was begun in November, 1922, when the calves were all approximately one hundred days old. During the following winter careful and numerous observations were made with the expectation that early signs of scurvy might be detected by noting the appearance and action of the calves. About January 26 signs of stiffness were noticed in the control calf E-2 and in the experimental calf E-3. Later in the winter all four of them showed

abnormal stiffness of the joints. This stiffness cannot be attributed to a deficiency of the antiscorbutic vitamin, however, for two reasons: First, the stiffness occurred in the control calves receiving the tomato juice as noticeably as it occurred in the experimental calves; and second, the stiffness disappeared when cod liver oil was substituted for butterfat in the ration, and never occurred during the second trial when the ration was
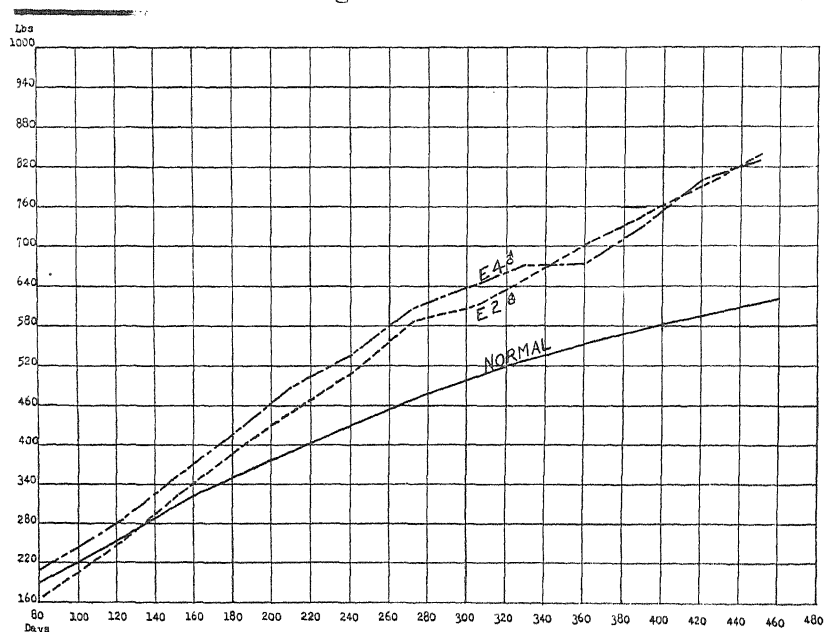
Fig. 3. Growth Curves of Two of the Calves Used in Experiment 1

E-4 received a vitamin C deficient ration while E-2 received the same ration supplemented by tomato juice as a source of vitamin C.

supplemented by cod liver oil and calcium carbonate. The growth of the experimental calves was fully equal to that of the controls.

Figures 2 and 3 give a comparison of the growth curves of the experimental calves and the controls with the normal growth curve. Figures 4 and 5 show two of the calves as they appeared at the close of the experimental feeding.

Autopsies were made on both of the experimental calves and

on one of the controls at the close of the experiment. These autopsies failed to show the lesions characteristic of scurvy. Following are the autopsy statements taken:

E-2 (control). Gums normal; teeth solidly imbedded in jaw; ankles and hocks normal. An abnormal amount of sinovial fluid and grease was found between the skin and flesh and between bone joints. The lymph glands and intestines were normal, no hemorrhagic areas being found. Small, nearly indistinguishable hemorrhagic spots were
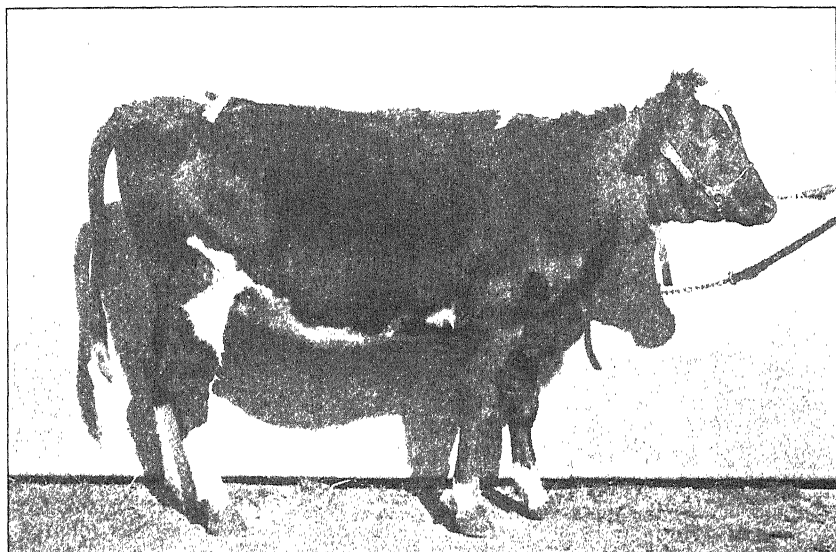
FIG. 4. CALF E-1 AT THE AGE OF 458 DAYS AFTER 364 DAYS ON EXPERIMENT

This animal received a vitamin C deficient ration supplemented by tomato juice. As might be expected she grew normally in every respect.

noticed at the costochondral junctions. The general condition of the carcass was pronounced normal both by the attending veterinarian and by the instructor in meats in the Agricultural College.

E-3 (experimental). Gums normal; teeth solidly imbedded in jaw; knee joints larger than normal but not hemorrhagic; hock joints had hemorrhagic spots 2 inches in diameter on each side. An abnormal amount of sinovial fluid was found in the joints. Intestines and lymph glands were normal and showed no hemorrhagic areas. The costochondral junctions were hemorrhagic and two of them were slightly

swollen. The carcass was pronounced normal except for the hemorrhagic areas noted above.

E-4 (experimental). Gums normal; teeth solidly imbedded in jaw; ankles normal; slightly distinguishable hemorrhagic areas on hock joints. A large abscess was found on the liver. The lymph glands of the intestines were hemorrhagic and slight hemorrhagic areas were noted on the costochondral junctions but no swellings could be found.

FIG. 5. CALF E-4 AT THE AGE OF 445 DAYS AFTER 365 DAYS OF EXPERIMENTAL FEEDING

This animal grew normally on a ration extremely low in its vitamin C content. Guinea pigs on the same ration die of scurvy in less than thirty days.

At the close of the experiment the control calf, E-1, was pregnant and was changed to the vitamin C deficient ration which she received for the three following months until she dropped her calf. This was done in order to secure a calf for a second trial that would have its body reserve of the antiscorbutic factor as low as possible. E-1 was slaughtered soon after calving, and some rather surprising abnormalities were found when her carcass was examined. All her costochondral junctions were

so greatly enlarged that they were noticeable as soon as the chest cavity was opened.   Suspecting that these enlargements were characteristic of rickets rather than of scurvy several of the enlarged junctions were examined histologically by Dr. Kernkamp and through his courtesy the report which follows is available.

Histologically the section shows hyperplasia of osteoid tissue in certain areas.   There is a proliferation of dense white fibers and also there are irregular masses of cartilage tissue with cartilagenous cells arranged in little groups or nests surrounded by a hyaline matrix. Marrow spaces were filled with white fibers, marrow cells and some blood vessels.   However, the zone of calcification could be seen and was regular, but the band of proliferative cartilage was irregular.

Dr. Kernkamp believed the bone showed signs of rickets but hesitated to definitely pronounce it as a distinct case of rickets.

Experiment 2

The majority of the evidence accumulated as a result of the first experiment indicated that calves either have no vitamin C requirement or that the amount needed is so low that it cannot be measured by feeding trials with guinea pigs.   However, the presence of a few of the scurvy-like lesions in the bodies of the experimental animals at the end of the trial as previously noted left some possibility of doubt as to the truth of such a conclusion. Consequently a second trial was begun in which it was the aim of the investigators to create more severe conditions relative to the deficiency of vitamin C, as well as to protect more efficiently against rickets by alterations in the ration.

In creating more severe conditions in this experiment calves less than two weeks old were placed immediately under conditions which would minimize the possible storage of the anti-scorbutic factor.   One of the calves, E-39, was dropped by E-1, which as previously mentioned, had for three months previous received a ration very deficient in vitamin C.   By this means it was expected that the possibility of a reserve of that factor

would be considerably lowered. Three other calves were obtained from a grade cow market near the college.

The ration used in this trial varied in two particulars from that used in the previous one. A mixture of timothy and wild hay was used in this case in place of the oat straw. No butterfat was fed in this trial but a drench of cod liver oil was used as a source of vitamin A and the antirachitic factor. The grain mixture was the same as previously used.
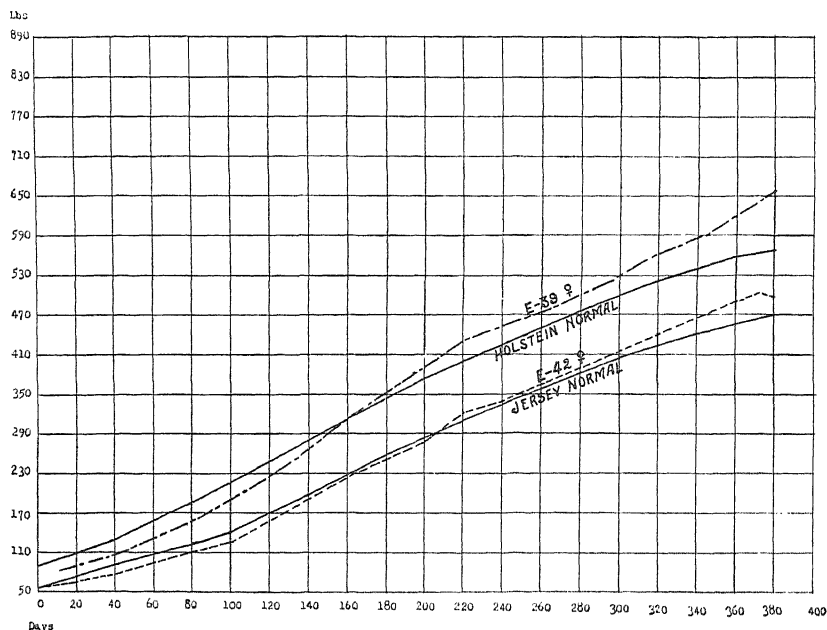
FIG. 6. GROWTH CURVES OF THE CALVES USED IN EXPERIMENT 2

E-39 received a vitamin C deficient ration while E-42 received the same ration supplemented by tomato juice.

The timothy and wild hay was bought in a quantity sufficient to last throughout the entire year. A representative sample of this hay was taken and fed *ad libitum* to guinea pigs along with the grain ration. All of the guinea pigs died of scurvy within three weeks. The other portions of the ration had already been tested in the previous trial and were known to be deficient in the antiscorbutic vitamin.

As in the previous trial the experimental feeding was carried on over a period of one year. At no time during this trial was stiffness noted in any of the calves. A glance at the growth curves (figs. 6 and 7) shows that all calves made normal growth and again the growth of the calves on the deficient ration was slightly better than that of the controls. Figures 8 and 9 show two of the calves as they appeared at the end of the experimental feeding.
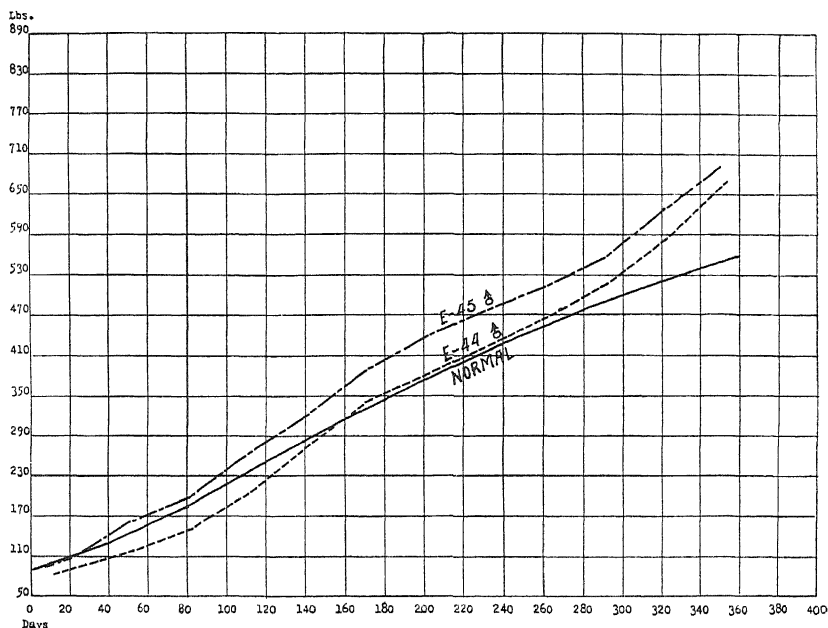
Fig. 7. Growth Curves of Two of the Calves Used in Experiment 2

E-45 received a ration deficient in vitamin C while E-44 received the same ration supplemented by tomato juice as a source of vitamin C.

E-39 (experimental) is now pregnant and will not be slaughtered for autopsy. E-42 (control) was not slaughtered for autopsy. E-44 (control) and E-45 (experimental) were slaughtered, however, and the following autopsy findings were reported:

E-44 (control). Perfectly normal throughout except for excessive amount of oily fluid at joints. Ribs, teeth, gums, and internal organs were all normal.
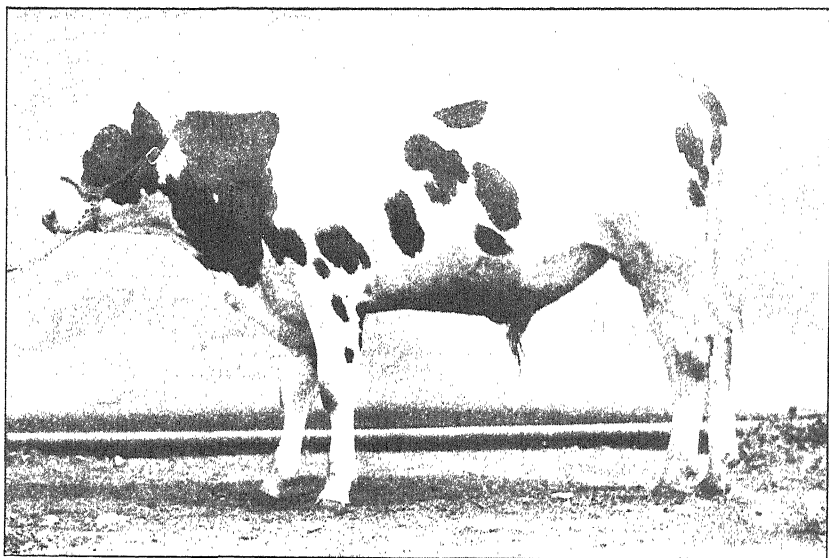
Fig. 8. Calf E-44 at the Age of 367 Days after 365 Days on Experiment

This animal received a ration deficient in vitamin C supplemented by tomato juice as a source of this vitamin. As might be expected this calf grew normally in every respect.
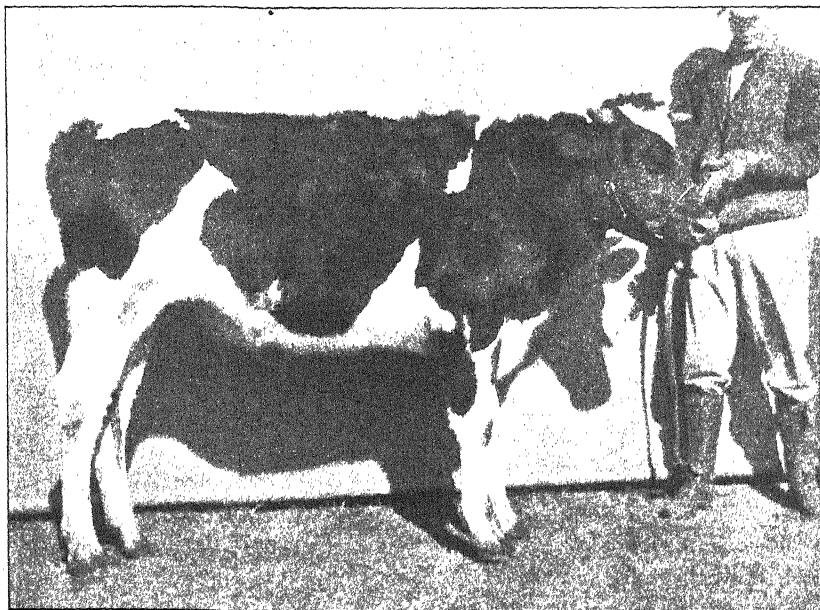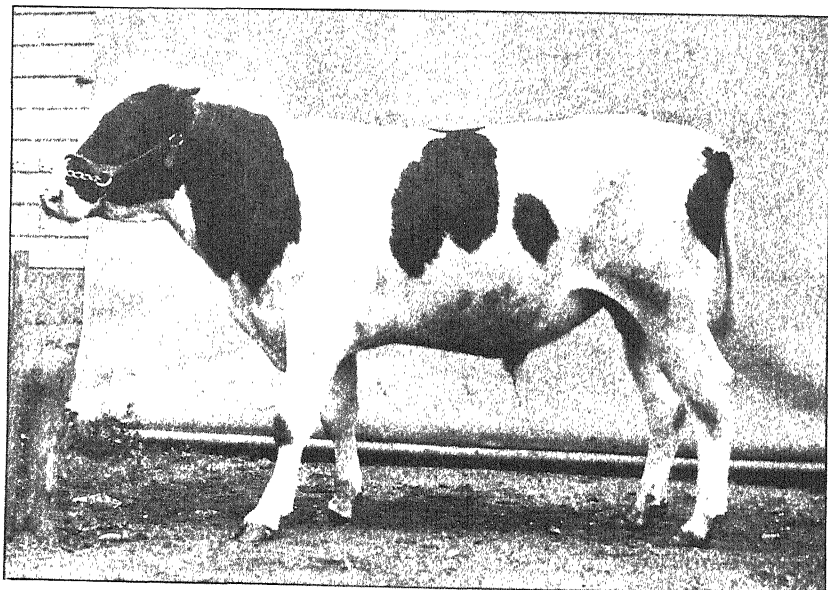

Fig. 9. Calf E-45 at the Age of 366 Days after 350 Days on Experiment

The animal grew normally on a ration extremely deficient in vitamin C. Guinea pigs on the same ration die of scurvy in less than thirty days.

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E-45 (experimental).   Perfectly normal except for oiliness at joints. The spleen was slightly hemorrhagic but all other organs, teeth, joints, and costochondral junctions were free from hemorrhagic areas and swelling.   The calf did not have scurvy.

These results indicate that the stiffness noticed in the calves in trial 1 and the hemorrhagic areas and swellings found on autopsy may be attributed to a deficiency of minerals or to a deficiency of the antirachitic factor rather than to a deficiency of the antiscorbutic vitamin.

A study of the vitamin C content of the livers of these calves is now under way with a view of offering an answer to the question, does the bovine synthesize vitamin C or does it have absolutely no requirement for this factor?   These results will be reported at a later date.

CONCLUSION

Calves do not require vitamin C in quantities that can be measured by the present method of testing food materials for their antiscorbutic potency by feeding them to guinea pigs.

Under practical conditions, even where very poor feeding practices are followed, there is little if any reason to believe the well-being of the calf will be affected by a shortage of vitamin C.

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HUMIDITY EQUILIBRIA OF MILK POWDERS*

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The absorption of atmospheric moisture by milk powders is a matter of no little consequence in its effect upon the keeping qualities of the product during storage. The preservation of its initial collodial structure and the prevention of the manifestation of the characteristic stale taste and odor and darkening of the color is to a large extent dependent upon the degree of success which can be attained in preventing the absorption of moisture from the air. Supplee and Bellis (1) have shown that a progressive development in the insolubility of milk powder casein is brought about by a moisture content and time relationship. Fouassier (2) has also shown the same phenomenon to be the result of a moisture and temperature relationship. Holm and Greenbank (3) have submitted data indicating that moisture content is involved in the development of tallowyness in dry milk containing fat, as well as in the development of a characteristic stale condition. These investigators state that a low moisture content is particularly favorable for the development of the tallowy condition, whereas a high moisture content causes a characteristic stale taste and odor defect described as fishy. These observations have been confirmed by the author (4) although the stale non-tallowy condition has in no instance been recognized as fishy.

The general agreement, even from limited data, as to the importance of moisture content of dry milk as a factor affecting its keeping properties during storage, warrants definite information showing the degree to which moisture may be absorbed under natural conditions. Such data have been obtained and are reported herein as representing substantially the humidity equilibria at various vapor pressures. The data are not presented as ab-

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* Received for publication December 23, 1924.

solute humidity equilibria values because it appeared that certain samples would not reach stability for several months and possibly not until after several years.   Therefore, in view of the normal periods during which milk powder is held, practical considerations were taken into account.   In no case were records kept for longer than six months and in numerous instances where equilibrium had apparently been reached the final record was obtained after a much shorter period.   The final data for those samples which had not reached constant weight were recorded only after two successive weighings two weeks apart showed a fluctuation of 0.05 per cent or less.

The methods described by Wilson (5) and Wilson and Fuwa (6) were used, but since information regarding the time required to reach equilibrium was desired, the aspiration method described by these writers was used only as a check method for a few samples.   Since close agreement with the longer holding method was obtained, the results are not duplicated herein.   Varying percentages of sulphuric acid solutions were used to obtain a range of vapor pressures corresponding to relative humidities from 10 to 80 per cent at 25°C.   Approximately two grams of milk powder were placed in small vials and held over the different acid mixtures in tightly sealed Mason jars.   Powders prepared by six different methods of manufacture, as well as powders containing different amounts of fat, were used for the determinations.   All samples, when placed at the various humidites contained less than 2.5 per cent moisture and were representative of fresh normal stock unless otherwise noted.   Weighings were made at short intervals during the first four weeks and at two week intervals thereafter.   After hydration at the different humidities had been completed, or practically so, all samples at humidities lower than 80 per cent were placed in an atmosphere corresponding to this vapor pressure and allowed to reach equilibrium which in no case required more than three weeks.   The samples were then returned to the respective humidities at which they had been previously hydrated.   The purpose of this procedure was to determine whether the curve of dehydration corresponded to the hydration curve.   As will be shown hereinafter marked differences were found.

TABLE 1

*Humidity equilibria of skimmed milk powders**

HUMIDITY	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4		SAMPLE 5		SAMPLE 6		AVERAGE ALL SAMPLES	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2
10	1.24	3.77	1.38	2.56	1.27	2.75	1.15	2.65	1.11	2.58	0.83	3.01	1.14	2.91
20	2.56	5.23	2.70	4.01	2.63	4.15	2.39	4.00	2.31	3.92	1.79	4.46	2.35	4.32
30	4.07	6.31	4.21	5.14	3.98	5.18	3.79	4.93	3.56	4.97	3.05	5.51	3.74	5.37
40	6.09	7.18	6.26	6.09	5.63	6.09	5.62	5.70	5.08	5.82	5.00	6.39	5.61	6.23
50	6.53	8.06	5.35	7.08	5.00	7.09	5.26	6.58	5.69	6.73	5.80	7.30	5.72	7.15
60	8.71	9.20	7.96	8.29	6.99	8.25	7.07	7.62	7.38	7.80	7.69	8.38	7.76	8.26
70	11.10	10.73	10.80	9.90	9.34	9.80	9.76	9.05	9.49	9.25	9.88	9.79	10.21	9.74
80	14.49	14.49	14.97	14.97	13.29	13.29	13.31	13.31	13.01	13.01	13.18	13.18	13.79	13.79

* Column 1 gives the hydration figures and column 2 the dehydration figures.

Table 1 shows the moisture content of six different brands of skimmed milk powder, each representing a different method of manufacture.   Sample 1 represents a product made by the Just double cylinder process in which the milk is not pre-condensed. Sample 2 represents the product from a single roller drum process in which low drying temperatures are used and in which the milk
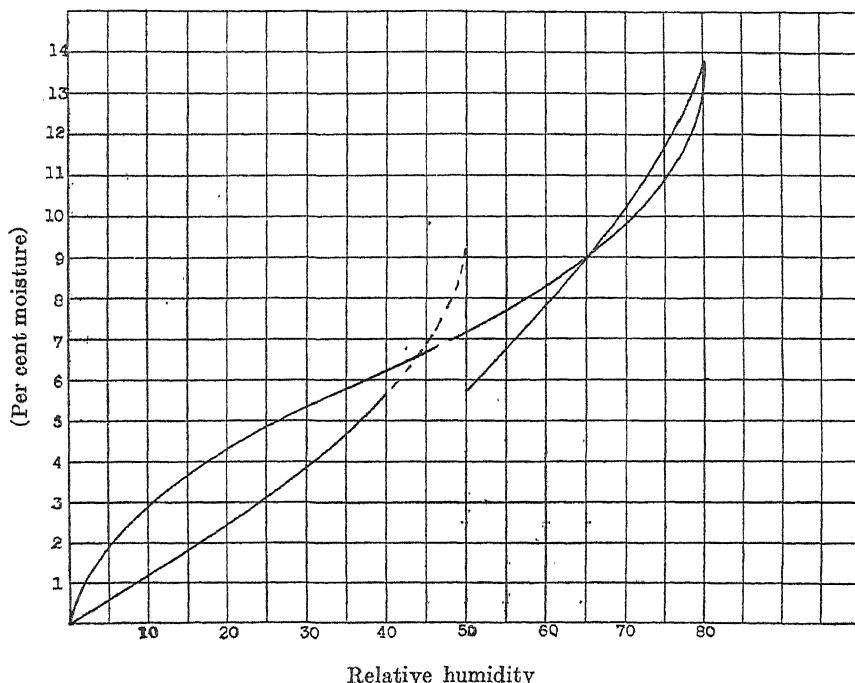
CHART 1. MOISTURE-HUMIDITY EQUILIBRIUM CURVES FOR SIX SKIMMED MILK POWDERS (TABLE 1)

The broken curve is hydration and the smooth curve is dehydration.   The dotted section indicates the area of greatest instability.

is previously condensed.   Sample 3 represents a spray process product in the manufacture of which unusually high initial temperatures are used.   The milk is not pre-condensed.   Sample 4 represents a product from a European centrifugal spray method in which the milk is precondensed.   Sample 5 represents the product from a spray method extensively used in this country

and for which the milk is previously condensed. Sample No. 6 represents the product from a still different type of spray process employed in this country but for which the milk is not pre-condensed. The results given in table 1 are the averages obtained from triplicate samples.

Chart 1 is constructed from the average results obtained from the different samples of skim milk powders recorded in table 1.
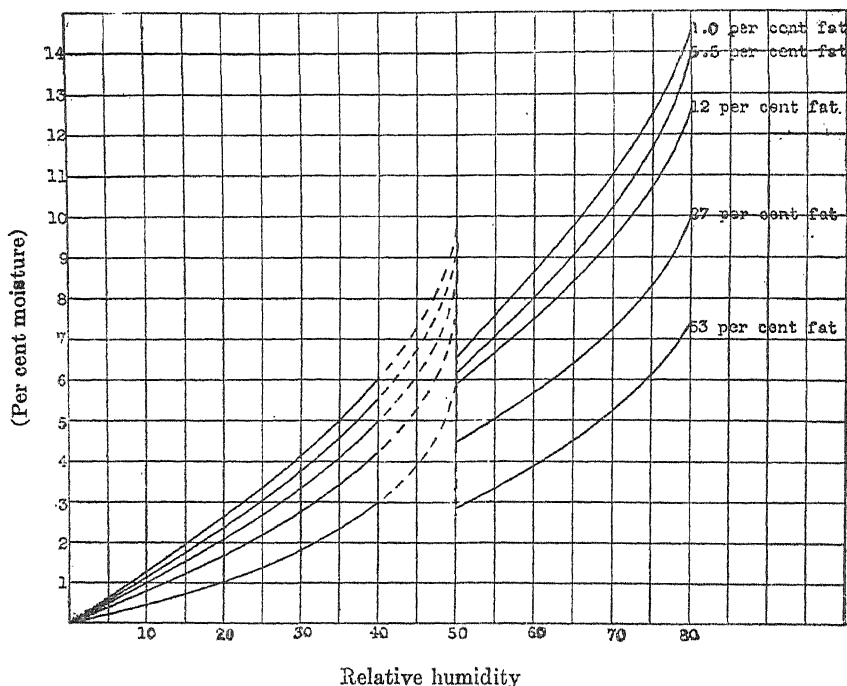
CHART 2. MOISTURE-HUMIDITY HYDRATION EQUILIBRIUM CURVES FOR JUST PROCESS MILK POWDERS WITH VARIABLE FAT CONTENTS (TABLE 2)
The dotted sections indicate the area of greatest instability

Table 2 shows the average results obtained from duplicate samples of Just Process powder containing variable amounts of fat. Charts 2 and 3 are constructed from the data in table 2, chart 2 showing hydration curves and chart 3 showing dehydration curves for the same powders. All results are reported on the basis of dry weight of the powder.

The results obtained show that the vapor pressure equilibria of milk powders involves certain features of peculiar interest. The characteristic type of the hydration curve and especially the break in this curve at approximately the same level for all powders of the same protein content, irrespective of the method of manufacture, but at different levels for powders with different
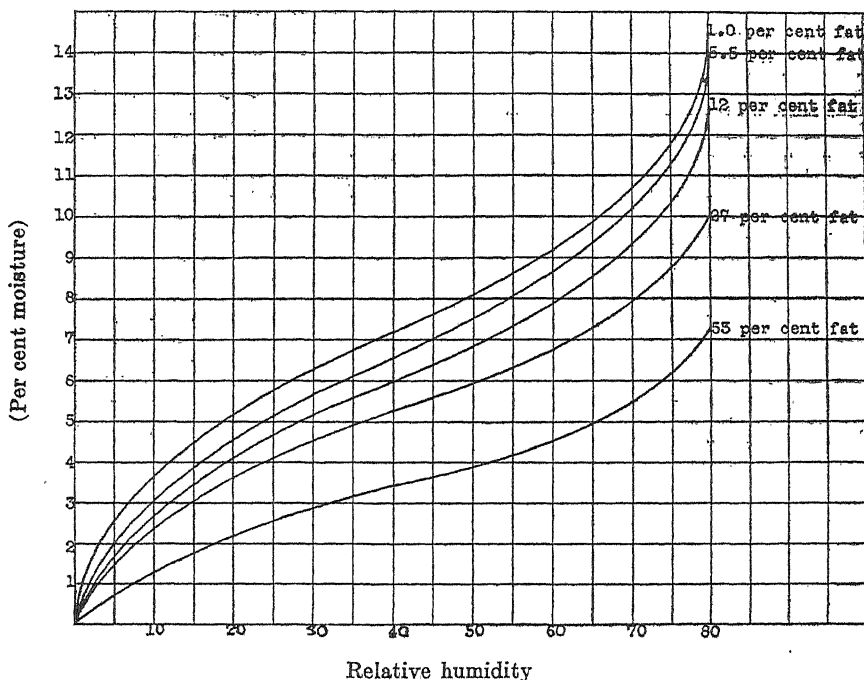
CHART 3. MOISTURE-HUMIDITY DEHYDRATION EQUILIBRIUM CURVES FOR JUST PROCESS MILK POWDERS WITH VARIABLE FAT CONTENT (TABLE 2)

protein contents, appears to involve the application of a fundamental principle governing moisture absorption by certain collodial materials. (Chart 1 shows only the compositve curve for all skimmed milk powders, but if each powder had been charted separately the same phenomena would be revealed in each instance.) Special significance lies in the interpretation of the practical aspects of the results in that the maximum moisture content at 50 per cent humidity was found by actual measurement

to have been reached within a few hours. The rate of decrease, however, after the maximum had been reached and until constant weight was obtained, was very slow as illustrated by the fact that in practically all instances decreasing weights were recorded

TABLE 2

*Humidity equilibria of Just Process milk powders with variable fat content**

HUMID-ITY	1 PER CENT FAT IN POWDER		5.5 PER CENT FAT IN POWDER		12 PER CENT FAT IN POWDER		27 PER CENT FAT IN POWDER		53 PER CENT FAT IN POWDER	
	1	2	1	2	1	2	1	2	1	2
10	1.24	3.77	1.12	3.13	0.91	2.75	0.77	2.33	0.42	1.31
20	2.56	5.23	2.41	4.61	2.00	4.14	1.69	3.59	1.01	2.22
30	4.07	6.31	3.75	5.70	3.31	5.18	2.83	4.51	1.79	2.94
40	6.09	7.18	5.67	6.61	4.98	6.02	4.32	5.25	3.04	3.42
50	6.53	8.06	6.20	7.58	5.88	6.87	4.48	5.90	2.85	3.96
60	8.71	9.20	7.92	8.69	7.50	7.90	5.61	6.72	3.88	4.51
70	11.10	10.73	10.28	10.20	9.47	9.29	7.20	7.92	5.23	5.42
80	14.49	14.49	14.00	14.00	12.68	12.68	10.01	10.01	7.37	7.37

* Column 1 gives the hydration figures and column 2 the dehydration figures.

TABLE 3

Rate of absorption and release of moisture by skimmed milk powder in an atmosphere with 50 per cent humidity

TIME	PER CENT MOISTURE	TIME	PER CENT MOISTURE
1 hour	4.26	18 hours	8.76
2 hours	5.19	30 hours	9.40
3 hours	5.98	33 hours	9.45
4 hours	6.71	38 hours	9.42
5 hours	7.07	53 hours	9.21
6 hours	7.36	65 hours	8.91
8 hours	8.02	9 days	7.88
10 hours	8.19	19 days	6.61
12 hours	8.35	28 days	6.51
14 hours	8.58	42 days	6.41
16 hours	8.68	56 days	6.38

at this humidity for a period of two to three months. An illustration of the rate of moisture absorption and subsequent release at 50 per cent humidity for one of the skimmed milk powders is shown in table 3. It is to be noted that absorption was very rapid during the first few hours and that the maximum was at-

tained after thirty-three hours. Thereafter there was a gradual decrease extending over a period of eight weeks before constant weight was reached. Rapid absorption in excess of the final equilibrium capacity was also noted at humidities of 60 and 70 per cent. The range, however, between the maximum and the point at which equilibrium was finally established, was not as great as at 50 per cent. The records from powder held at 40 per cent humidity or lower showed continuous absorption, or only slight dehydration depending upon the initial moisture content, to the point designated as equilibrium.

TABLE 4

Comparison of humidity equilibria of skimmed milk powder subjected to various treatments *

HUMIDITY	FRESH SAMPLE NO TREATMENT		SAMPLE HEATED TO 100°C. IN VACUO FOR ONE HOUR		SAMPLE ALLOWED TO ABSORB 10 PER CENT MOISTURE AND THEN DRIED OVER P_2O_5 AT 50°C.	
	1	2	1	2	1	2
10	1.24	3.77	0.75	2.50	0.45	0.78
20	2.56	5.23	1.57	3.70	0.92	1.49
30	4.07	6.31	2.71	4.57	1.58	2.16
40	6.09	7.18	4.18	5.20	2.41	2.89
50	6.53	8.06	4.67	6.02	3.71	3.80
60	8.71	9.20	6.70	7.51	4.28	4.59
70	11.10	10.73	9.30	9.80	5.27	6.38
80	14.49	14.49	13.79	13.79	10.99	10.99

* Column 1 shows the hydration figures and column 2 the dehydration figures.

In contrast with the hydration results it is to be noted that the dehydration curves represent typical absorption curves. While various possibilities might be suggested as causing these differences, as well as in explanation of the characteristic hydration curves, it is believed that the most tenable explanation involves application of the general laws of capillarity as applied to collodial matter. In this connection it is to be noted, however, that nearly three weeks were required for saturation at 80 per cent humidity before the samples were again returned to the lower humidities for dehydration. Since it has been previously shown (1) that high moisture and time are factors which bring about marked changes in the properties of milk powder casein, it is

probable that these samples at the beginning of dehydration possessed properties of an entirely different character than before hydration. The phenomenon of hysteresis may therefore be involved. Although the question of insufficient time for establishing complete equilibrium is not entirely excluded, this factor is considered to be irrelevant for all practical considerations. The
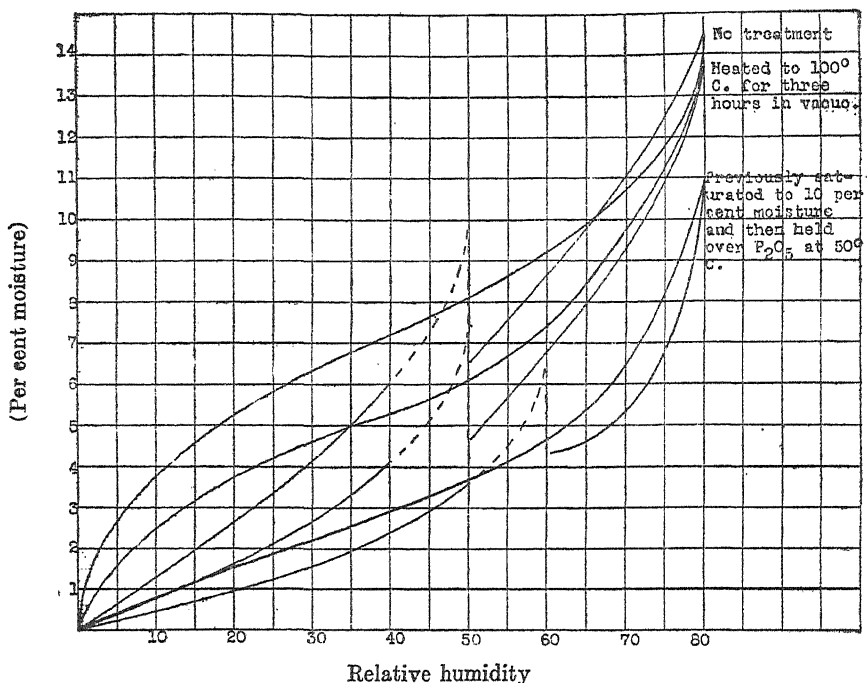
CHART 4. MOISTURE-HUMIDITY EQUILIBRIUM CURVES FOR JUST PROCESS SKIMMED MILK POWDER SUBJECTED TO PREVIOUS HEAT TREATMENT AND MOISTURE SATURATION (TABLE 4)

The broken curves are hydration and the smooth curves are dehydration. The dotted sections indicate the areas of greatest instability.

possiblity of hydration of anhydrous lactose has been considered, and while it is known that such a change does take place in milk powder of high moisture content after prolonged storage, the information obtained in connection with this work indicated that the anhydrous form was not completely converted to the stable monohydrate.

It is well known that the absorptive properties of collodial matter may be materially altered by the conditions to which it is subjected. It is therefore deemed desirable to include data showing the degree to which the absorptive properties of milk powder may be affected by certain treatments. A sample of skim milk powder made by the Just process, and for which the absorption data has already been recorded, was divided into two parts and treated as follows: One part was dried in vacuo at 100°C. for one hour and then subdivided and held at various humidities as heretofore described. The second part was placed in a saturated atmosphere until it had absorbed 10.53 per cent moisture. It was then immediately placed over phosphorus pentoxide and dried at 50°C. When completely dried it was subdivided and placed at the different humidities already indicated. The hydration and dehydration results are shown in table 4 and chart 4. In comparing these records with those obtained from the normal skimmed milk powder of the same composition it is to be noted that the same general type of curves were obtained. In both instances, however, lower equilibrium levels were found. Furthermore, the results obtained from the saturated sample subsequently dried at a low temperature were lower than those obtained from the sample heated to a high temperature but which had not previously contained a high moisture content.

These results from the artificially treated milk powder are probably of little value for practical interpretation but nevertheless they serve to illustrate the effect of heat treatment and previous moisture absorption on the subsequent ability of such products to retain moisture at different vapor pressures. It would be of interest to study the effects of analogous relationships as they apply to milk powders subjected to different heat treatments during the process of manufacture.

While it has been impossible to include data showing the absorption of moisture at humidities above 80 per cent due to difficulties from mold formation, records were obtained for a limited period wherein the absorptive capacity of skimmed milk powder in a saturated atmosphere is shown in a striking manner.

The particular sample in question absorbed 6.25 per cent of its dry weight during the first half hour of exposure. At the end of four hours the moisture content was 15.49 per cent; after twenty-five hours it was 27.25 per cent; and after one hundred hours a moisture content of 51.11 per cent was recorded.

It is not to be inferred that the data recorded in this paper can be applied directly to any particular lot of milk powder held at the given humidities. These results represent maximum absorptive capacity. As a rule lower moisture contents of commercial samples will be found, because of the variable degrees of protection which the container affords and because of slow diffusion through the mass. It is not uncommon, however, to find a moisture content 4 to 5 per cent higher in the outside areas of a barrel of milk powder than is found at the center.

SUMMARY

1. Data are given showing the moisture content of various milk powders at equilibrium in vapor pressures corresponding to humidities from 10 to 80 per cent at 25°C.

2. It is shown that the absorption of moisture by milk powder irrespective of the method of manufacture, apparently proceeds according to certain fundamental laws governing absorption by collodial matter.

3. The data show that at humidities above 50 per cent the initial moisture absorption is very rapid, and at humidities between 50 and 70 per cent this initial absorption is not retained by the powder. When the maximum is reached a dehydration process begins and extends over a period of months before equilibrium is established at a lower level.

4. The dehydration results obtained after milk powder had been subjected to a relatively high moisture content for a period of three weeks, show a different moisture retention at the various humidities than was shown during hydration.

5. The absorptive properties of milk powder were found to be altered by excessive heating and previous saturation.

6. Milk powders with variable fat content are shown to possess different capacities for moisture absorption and retention. The

absorptive ability decreases with increased fat content and correspondingly lower protein content.

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SWEETENED CONDENSED MILK

III. IN A TOTAL SOLIDS RESIDUE WHAT IS THE FORM OF LACTOSE?*

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Where reference is made to lactose in the literature there is much confusion as to which is meant—the hydrate or anhydrous form. This sugar under ordinary conditions crystallizes as $C_{12}H_{22}O_{11} \cdot H_2O$ and this is the form in which it appears on the market and in the laboratory generally. In many cases, however, calculations are based on a molecular weight corresponding to $C_{12}H_{22}O_{11}$, though perhaps generally the molecule of water is taken into consideration. For instance, the figure 52.5 is given in most texts as the specific rotation of lactose; this is calculated on the basis of the hydrate. In quantitative data there is, of course, a difference of 5 per cent depending on which form is used.

Lactose is sometimes determined in condensed milk by subtracting the sum of the other solids from the total dry matter; in this case it is important to know whether the hydrous or anhydrous form is present in the dry matter. But this work was carried out particularly because it was necessary that this point be cleared up before some of the calculations could be made that are involved in the next article of this series.

It is known that lactose hydrate is very stable, the molecule of water being driven off only at about 130°C. On the other hand, data has been presented which shows that the evaporation of an aqueous solution of lactose above 93°C. yields the anhydrous

form (1). In the determination of total solids in condensed milk, usually a water solution is evaporated on a water or steam bath, or hot plate. It would be expected, therefore, that the conditions would be right for the production of the anhydrous form.

Two experiments were carried out: In one an aqueous solution of pure lactose was evaporated and the residue dried under conditions similar to those observed in the total solids determination. In the other, a known amount of lactose was added to a condensed milk solution and the determination carried out in the same way; this result compared with the solids residue obtained from the condensed milk alone gave data from which could be determined the condition of the lactose in the residue.

The lactose used in these experiments was of highest purity containing 0.2 per cent hygroscopic moisture. It showed the theoretical specific rotation.

The procedure of the A. O. A. C. for the determination of dry matter in sweetened condensed milk was followed in all cases, employing ignited sea sand for drying residues.

EXPERIMENT 1

Two lactose solutions were prepared containing (a) 5 grams and (b) 10 grams in 100 cc. Ten cubic centimeter portions of these solutions were carried through the total solids determination in quadruplicate. The following weights of residue were obtained:

Solution 1. 0.4721, 0.4722, 0.4728, 0.4721 gram.
Solution 2. 0.9449, 0.9452, 0.9459, 0.9469 gram.

Since the lactose used contained 99.8 per cent $C_{12}H_{22}O_{11}$, there should have been in the first set of residues 0.4740 and in the second 0.9481 gram if the anhydrous form were present. These figures are very close to those obtained experimentally. The experimental results being somewhat lower than the theoretical is perhaps to be accounted for by a slight decomposition of the lactose while drying.

EXPERIMENT 2

Solutions containing 20 grams fresh condensed milk in 100 cc. were prepared (M). Two solutions of lactose containing 5 grams (A) and 10 grams (B) in 100 cc. were used as a convenient means of adding the lactose. The results obtained are given in table 1.

TABLE 1

	WEIGHT OF RESIDUE	
	(1)	(2)
	grams	grams
10 cc. M + 10 cc. A............................	1.9336	1.9398
10 cc. M.......................................	1.4543	1.4633
Difference....................................	0.4793	0.4765
10 cc. M + 10 cc. B............................	2.4136	2.4189
10 cc. M.......................................	1.4543	1.4633
Difference....................................	0.9593	0.9556

Since the actual added lactose ($C_{12}H_{22}O_{11}$) in the first series was 0.4740 gram and in the second 0.9481 gram, it is clear that in the process of evaporating and drying, nearly all the sugar appeared in the residue in the anhydrous form. Where the smaller addition was made (solution A) the anhydrous state was more nearly reached than with the larger addition. It may be concluded, therefore, that in condensed milk where the content of lactose is still less, this sugar appears in the total solids residue in the anhydrous form.

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THE INFLUENCE OF THE PERIOD OF HEAT ON MILK PRODUCTION

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It is generally believed that oestrum, or the period of heat has a depressing influence on the production of dairy cows. However, there is little direct evidence by which the validity of this can be tested.

RÉSUMÉ OF PREVIOUS WORK

It was reported by Hooper (1) that in 54 records obtained from cows kept for three years that the oestral period caused a considerable variation in production with some cows while with others it seemed to have but little effect. Later Hooper and Brown (2, 3) found from the records of 29 cows that there was an average decrease of 0.1 pound in fat and 1.5 pounds in milk production on the day of most evident heat. Some cows showed an increase in production however while others showed no variation.

DATA PRESENTED

The data summarized and presented in table 1 were collected during the years 1907 to 1921 inclusive from the herd at this station. Not all heat periods are included, as it was felt best to collect the records from the dates of breeding only. Consequently the day on which a cow was bred was looked on as the day of most evident heat and the average milk production on the days of most evident heat was obtained and compared with the average production on each of the three days preceding and following it. In all a summary of 868 cases is given.

It will be noticed that on each of the three days preceding and following the days of breeding the average milk production is higher than on the day of breeding; the highest average production being two days before breeding while the others ran at an apparently normal rate with the exception that on the day follow-

ing the date of breeding the production was also somewhat lower
than on the other days included.

TABLE 1

Influence of oestrum on production

GROUP	NUMBER ON COWS	NUMBER OF HEAT PERIODS	DAYS BEFORE AND AFTER BREEDING WITH AVERAGE PRODUCTION						
			3	2	1	0	1	2	3
			pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
Holsteins..........	28	215	30.9	31.2	30.8	30.1	29.8	30.6	30.7
Jerseys............	32	198	18.0	18.2	18.2	17.7	17.8	18.2	18.1
Guernseys.........	28	155	20.3	20.6	20.3	19.9	20.3	20.5	20.6
Ayrshires..........	14	72	25.2	25.6	25.2	24.8	24.6	25.0	25.2
Scrubs............	8	49	14.9	15.4	15.2	14.7	14.7	14.9	15.0
First Grades.......	16	80	17.0	16.6	17.0	16.3	16.5	16.7	16.9
Second Grades.....	19	99	16.8	16.5	16.7	15.8	15.9	16.4	16.7
Total............	145	868	21.8	22.0	21.8	21.2	21.3	21.7	21.8

TABLE 2

Variations in the influence of oestrum on production in the case of Holstein cow 44

DAYS BEFORE AND AFTER BREEDING	MILK PRODUCTION IN PERIODS		
	I	II	III
	pounds	*pounds*	*pounds*
3	38.5	35.6	38.7
2	37.9	34.5	40.2
1	39.0	38.1	42.1
0	37.6	40.4	41.5
1	43.5	42.1	40.1
2	42.3	42.2	43.6
3	44.3	39.5	43.6
Average for 6 days before and after breeding..	40.9	38.7	41.5
Percentage income over day of breeding....	−8	4	0

This increase in production on the second day before breeding
as compared with that on the day of breeding is only 4 per cent
for the total group considered.   This is not a large variation but

when it is considered as being from a large number of animals, and that a similar increase occurs in each group of the animals then it is perhaps safe to deduce that there is some reason for the production being high two days before breeding and low on the day of breeding and that following it as compared with the production on other days near that time.

One possible explanation is that as the period of heat starts some galactogogic hormone is produced in greater quantities, either as a purely incidental process, or in an effort to stimulate production at this time so as to compensate for the lowered production two days later.

Though it appears that there are fairly uniform changes in production around the period of heat in the case of large groups of cows, yet individual cows vary considerably and an individual cow may show wide variations in her behavior from one heat period to another.

This is well illustrated in the case of the Holstein cow 44 in the College Herd at three breeding dates.

In this case it will be found that the cow 44 showed all the variations possible in production at different times of breeding. This indicates variations in production at the time of heat which can not be accounted for.

SUMMARY

From the data presented here it would appear that:

1. There is a decrease in milk production on the day of breeding and the day following.

2. An apparently compensating increase takes place two days before breeding.

3. Individual cows show very wide differences in their production at this time.

4. Wide variations in behavior may be expected between different heat periods with the same individual.

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FACTORS INFLUENCING THE VISCOSITY OF CREAM AND ICE CREAM*

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It is a well known fact that pasteurization, aging, homogenization and the amount of binder used all have an influence upon the viscosity of the product. Pasteurization brings about a decrease in the viscosity of the cream or ice cream mix to such an extent that if the product were to be made into ice cream at once difficulty would be experienced in obtaining the desired overrun and the texture would be poor. This difficulty may be overcome by aging the cream or mix for twenty-four hours or longer.

Trade preparations, known as "improvers" are frequently used to hasten the ripening and bring about the desired viscosity in a few hours if necessary. The increase in viscosity brought about by the use of an "improver" is probably due, in some cases at least, to coagulation. However, when no "improver" is used the increase in viscosity is probably not due to coagulation, but is probably due to physico-chemical changes. If the whole mix is pasteurized and homogenized the increase in viscosity is due, in part at least, to the presence of the gelatine. However, the increase in viscosity brought about in pasteurized and homogenized cream is quite pronounced. Just what factors are responsible for this are not definitely known although several theories (1) have been advanced to explain this phenomenon.

According to one theory it is due to the knitting together of the colloidal particles in the cream thus restoring the viscosity. A second theory is that this increase in viscosity is due to the hydration of the proteins while held at the low ripening temperature. A third theory is that it is due to enzymatic action, while a fourth theory is to the effect that the increase in viscosity is due to a greater clumping together of the fat globules during aging.

The viscosity of cream generally increases with age. The rate of increase in viscosity of an homogenized cream is much greater than that of a cream pasteurized but unhomogenized held under the same conditions. The increase of viscosity in cream immediately after homogenization over the same cream before homogenization is due to the taking up or fixing of more of the milk serum. This is brought about by the breaking up of the fat globules resulting in an increase in the total surface area of the fat globules.

The microscopical appearance of the fat globules differ exceedingly from that of a pasteurized sample. The latter when examined under the microscope shows the globules spread out evenly over the field and separated from each other, although in some cases globules will be seen touching each other. The fat globules in the homogenized cream present an entirely different appearance. The globules are about one-tenth the original size and are clumped together in clusters, varying in size from very small clumps of three or four globules to large clumps of several hundred. This clumping of the fat globules suggested the idea that aging of the cream causes the fat globules to come together in larger clumps. If this is the case it is probable that the increase of viscosity is due to the enclosure of serum in these clumps. Bancroft (1) states that "of particles agglomerate loosely into spherical masses, the viscosity will increase because the water in the voids inside the spherical agglomerates no longer counts as free water."

The work herein reported represents an attempt to determine whether or not the clumps of globules did increase in size during a period of storage and if a high viscosity could be attributed to a greater grouping together of the fat globules.

THE INFLUENCE OF PASTEURIZATION, HOMOGENIZATION AND AGING UPON THE VISCOSITY OF CREAM

Methods

The cream used was sweet cream of less than 0.20 per cent acidity and standardized to 18 per cent butter fat with whole

milk. Pint samples of the cream were taken after pasteurization and immediately after homogenization. The viscosity of each sample was determined and a microscopical examination made of the fat globules. These samples were then placed in storage and examined at intervals of twenty-four hours.

It was found necessary to dilute the cream in order to examine it under the microscope. The following method, which could be closely adhered to from day to day, was adopted. Nessler tubes, graduated to hold 50 cc. were filled to the 50 cc. mark with distilled water. Two drops of each sample of cream were added to separate tubes from a 1 cc. pipette, the same pipette being used from day to day. The tubes were then corked tightly and inverted slowly and the mixture allowed to run to the opposite end of the cylinder. The cylinder was then brought back slowly to its upright position three times thus mixing the cream thoroughly through the water. A drop of this mixture was then placed on a slide for observation.

In one instance in the first part of this experiment the viscosities were secured with the Scott viscosimeter. The cream to be tested was brought to a temperature of 17°C. and 200 cc. transferred to the viscosimeter. The length of time required for 50 cc. of the cream to run through the aperture was determined by means of a stop watch. The specific viscosity was calculated by dividing the time viscosity by the time required for 50 cc. of distilled water at the same temperature to run through the viscosimeter.

The remaining viscosities were obtained with the Doolittle Torsion Viscosimeter. Two determinations were always made; one by rotating the wire to the right; the other to the left, and the average of the two readings taken.

A description of the field observed under the microscope was decided upon as the best method of keeping a record of the appearance of the clumps of fat globules.

Results secured

The data secured on the viscosity of the pasteurized cream and homogenized cream and the appearance of the fat globules under

the microscope are presented in table 1.   A study of the data in these tables reveals the fact that the viscosity of pasteurized cream shows very little increase during aging.   In five of the six trials the pasteurized cream had a slightly higher viscosity at the holding period than when fresh although there was not a steady increase from the time the first reading was taken.   The tendency seemed to be for the viscosity to remain the same or drop somewhat during the first twenty-four hours and then to increase slightly as the aging progressed.   The appearance of the fat globules in the pasteurized sample was practically constant in all the trials in that the globules were separate and did not change upon aging.   In experiment 2 the pasteurized cream when forty-eight hours old had a clump of three or four globules here and there on the microscopic field under observation.   The fact that no clumping of the fat globules was observed in the pasteurized sample does not prove that it did not occur.   The probability is that if clumps are formed the globules do not cling together as tenaciously as in homogenized cream and the mixing of the cream and water would break up these clumps if any were present.

The results obtained for the homogenized cream proved rather contradictory in that the viscosity did not always increase during aging.   The data obtained in experiments 1 and 2 show that there was a steady rise in the viscosity at the end of each twenty-four hours.   The data obtained in experiment 3 show a decrease in viscosity at the end of twenty-four hours but an extremely high viscosity after forty-eight hours.   The data secured in experiments 4 and 6 show a slow decline in viscosity from the fresh sample up to and including the seventy-two hour old sample. Experiment 5 shows a falling off in viscosity up to the end of the forty-eight-hour period and then an increase so that the reading taken at seventy-two hours was higher by 1.75° than when fresh. In general there was a direct relationship between the appearance of the fat globules and the viscosity.   That is, where the viscosities increased, the clumps of fat globules were larger, and where the viscosity decreased, the size of the clumps remained the same as when the sample was fresh.

TABLE 1

The influence of aging upon pasteurized cream and upon pasteurized and homogenized cream

EXPERIMENT		PASTEURIZED	HOMOGENIZED	PASTEURIZED	HOMOGENIZED	PASTEURIZED	HOMOGENIZED	PASTEURIZED	HOMOGENIZED
1	Specific viscosity	1.22	9.01	1.22	12.62	1.31	Would not run	1.23	Would not run
	Age............ Appearance of fat globules	Fresh Separate and spread over field	Fresh Several large clumps	24 hours Same as fresh	24 hours Many large clusters	48 hours No distinct clumping	48 hours Fat clumps large and numerous	72 hours No distinct clumping	72 hours Clumps very large and numerous
2	Viscosity in degrees retardation	7.00	110.00	6.75	126.25	7.25	Too viscous to get reading		
	Age............ Appearance of fat globules	Fresh Separate and spread over field	Fresh Globules clumped together	24 hours Same as fresh	24 hours Few large clumps	48 hours 1 or 2 clumps of 3 or 4 globules	48 hours Clumping increased clusters larger		
3	Viscosity in degrees retardation	9.5	169.00	9.25	110.00	10.00	345		
	Age............ Appearance of fat globules	Fresh No clumping of fat globules	Fresh Many clumps	24 hours No clumping	24 hours Many clumps				
4	Viscosity in degrees retardation	10.50	49.25	9.00	48.75	9.75	46.40	9.75	45.00
	Age............ Appearance of fat globules	Fresh Globules separate	Fresh Clumps very small	24 hours No change	24 hours Same as fresh	48 hours No change	48 hours No change	72 hours No change	72 hours No change

5 Viscosity in degrees retardation	9.50	142.75	9.50	140.75	9.50	129.50	10.50	144.50
Age............	Fresh	Fresh	24 hours	24 hours	48 hours	48 hours	72 hours	72 hours
Appearance of fat globules	Fat globules separate	Small cluster	Fat globules separate	Same as fresh	No change	No increase in size of clumps	No change	No change
6 Viscosity in degrees retardation	8.25	84.75	8.00	80.00	8.50	78.50		
Age............	Fresh	Fresh	24 hours	24 hours	48 hours	48 hours		
Appearance of fat globules	Globules separate	Medium sized clumps	No change	No change	No change	No change		

THE EFFECT OF AGITATION UPON VISCOSITY

Since the results obtained above indicate that with an increase in viscosity there is a greater clumping together of the fat globules to which can be attributed the higher viscosity, for the reason that as the clusters grow larger more serum is enclosed by them resulting in an increase in viscosity, it was decided to carry the work farther and attack the problem from another angle. During the freezing process the mix is subjected to violent agitation, which would naturally be expected to break up the clusters of fat globules. If the supposition that the clumping together of the fat globules increased the viscosity is correct then the reverse should also be true; namely, if the fat clumps are broken up the viscosity should decrease.

Methods

Samples were taken of homogenized cream after aging and of the mix before running it into the freezer. The freezer used in this work was a 40-quart brine freezer and the brine was made with ice and salt. A Manton-Gaulin homogenizer with a capacity of 60 gallons per hour was used for homogenizing.

The formula adopted for the ice cream was as follows:

42 pounds 18 per cent cream
8 pounds sugar
4 ounces gelatin
4 ounces vanilla

During the freezing process samples were drawn from the freezer at the end of two, four, six, eight, and ten minutes. These consecutive samples were examined under the microscope and their viscosities determined with a Doolittle Torsion Viscosimeter, after carefully warming to 17°C. to allow the enclosed air to escape.

In the microscopic examination seven Nessler tubes were used and the dilutions prepared as previously explained. A glass slide, large enough so that a drop of all seven dilutions could be arranged in sequence upon it, was used. This allowed of a rapid change under the lens from one drop to another and a more

TABLE 2

The influence of agitation upon the viscosity of cream and ice cream mix

EXPERIMENT		CREAM	MIX	WHIPPED 2 MINUTES	WHIPPED 4 MINUTES	WHIPPED 6 MINUTES	WHIPPED 8 MINUTES	WHIPPED 10 MINUTES
1	Viscosity in degrees retardation	141.75	92.75	24.50	18.50	18.00	16.50	14.50
	Appearance of fat globules	A few large clumps, many medium and small clumps	Few large clusters many medium and small clumps	3 or 4 large clusters, others small	No big clumps, many small clumps	A few small clumps, mostly individual globules	Only a very few, very small clumps	About the same on 8 minutes sample
2	Viscosity in degrees retardation		39.50	39.00	17.25	13.25	13.50	10.75
	Appearance of fat globules		No large clumps, many medium and small clumps	Fewer clumps, more individual globules	Clumps fewer and smaller	Practically no medium sized clumps but many small ones	Clusters very small, many individual fat globules	Scarcely a clump left
3	Viscosity in degrees retardation	154.00	89.00	21.00	Lost	16.25	12.50	10.50
4	Viscosity in degrees retardation	240.00	85.50	14.00	10.75	10.00	9.50	8.75
5	Viscosity in degrees retardation	154.50	33.25	14.25	11.25	10.75	10.50	9.25
6	Viscosity in degrees retardation	184.25	29.25	18.75	15.50	15.75	13.00	11.25
	Appearance of fat globules	The appearance of the clumps in the last four runs was similar to the other runs. The cream had large clusters present and as the viscosity decreased so did the size of the clumps until at the end of the ten minutes scarcely any clumps were to be seen in the field						

accurate comparison of the clumps in the different samples was possible.

Results obtained

The data secured on this part of the problem are presented in table 2.   The viscosity droped consistently in each sample taken with the exception of the eight-minute sample in experiment 2, where the viscosity of the eight-minute sample was 0.25 degree higher than that of the six-minute sample.   However, since the difference is so small and in all other cases there was a consistent fall in viscosity we are justified in concluding that there is in general a lowering of the viscosity brought about by agitation. The greatest drop was from the cream to the mix, the stirring necessary to mix the sugar, gelatine, and vanilla evidently being disasterous to the viscosity.   That such is the case should be borne in mind when preparing the mix and unnecessary stirring of the cream avoided.   If the whole mix is pasteurized and homogenized this factor is not so important.

SUMMARY

An effort was made to discover whether or not the increase in viscosity of cream during aging could be attributed to an increase in the size of the clumps of fat globules.   Samples of pasteurized and homogenized cream were collected and the viscosity taken every twenty-four hours, and at the same time a microscopic examination of the fat globules was made.   In three of the trials the viscosity increased during aging, in two it decreased and in the remaining one it was lower at the end of twenty-four hours and forty-eight hours but was slightly higher after seventy-two hours than when fresh.   The appearance of the clumps of fat globules seemed to bear a fairly definite relation to the viscosities, in that in those experiments where the viscosity increased, the size of the clusters apparently increased also, while in the trials where no increase was encountered the clumps showed no indication of becoming larger during the holding period.

The influence of agitation on the viscosity and the size of the clumps of fat globules was studied by collecting samples of the

aged cream, the same cream after the sugar, gelatine, and vanilla had been stirred in and of the mix after two, four, six, eight, and ten minutes agitation in the freezer. These samples were then examined under the microscope and viscosity determinations made. From the results obtained it is evident that agitation causes a marked reduction in the viscosity and that there is a corresponding reduction in the size of the cluster of fat globules.

CONCLUSIONS

1. Cream in some cases showed an increase in viscosity upon aging and in other instances did not. The clumps of fat globules were larger in those cases where there was a rise in viscosity.

2. Agitation caused a decided reduction in viscosity of cream, with an accompanying reduction in the size of the clumps of fat globules.

3. The results of this investigation indicated that the increase in viscosity, noted in cream during aging, is attributable to a greater grouping together of the fat globules with its attending fixation of a part of the free serum.

REFERENCE

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A STUDY OF CALCIUM AND PHOSPHORUS BALANCES WITH DAIRY CATTLE*

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The results of many years of study by numerous investigators and many practical aspects of the problem have developed an increasing interest in the utilization of calcium and phosphorus by dairy cattle.

The limited ability of the dairy cow to utilize the mineral constituents of various rations was demonstrated in series of experiments by Forbes (1). Normal dry winter rations were fed and variously included, timothy, clover or alfalfa hay and in some cases corn silage. Negative calcium balances were obtained in all cases where the daily milk production exceeded 10 pounds, the losses being less with clover hay and alfalfa than with timothy hay. In most cases the phosphorus balances were negative.

Hart and associates (2) reported an appreciable retention of calcium when alfalfa hay that had been cured under caps was fed to liberally milking cows. A more liberal storage resulted from the use of fresh green alfalfa. Positive phosphorus balances accompanied the storage of calcium. It was thought that the quality of the roughage determined the degree of calcium assimilation, by the term quality being meant the relative degree of destruction in the curing process of the unknown factors affecting calcium assimilation.

Steenbock and associates (3) reported from results obtained with rats that alfalfa cured in the dark was very rich in vitamin A, even 0.5 per cent supporting normal growth when the anti-

rachitic factor was supplied in the form of light, otherwise 4 per cent being required. When cured in the sun, exposed to dew and rain, vitamin A was destroyed but an antirachitic activation of certain substances in the alfalfa was suggested.

In later work Hart and associates (4) reported that negative calcium and phosphorus balances were obtained with liberally milking cows on alfalfa hay that had been cured in the windrows for four days. Greater losses were obtained on timothy hay. In further work (5) it was found that on rations including fresh green plant tissue, such as June grass and white clover, negative calcium balances were obtained. Inquiry into the previous nutritional history of the cows accounted for the negative balances and a repetition of this experiment taking into account this factor, showed that liberally milking cows were not maintained in calcium equilibrium on the character of green grasses fed unless a supplement of bone meal was added. In this experiment appreciable positive phosphorus balances accompanied losses of calcium.

Hart and associates (6) have more recently reported that upon daily exposure for twenty minutes to the emanations from a mercury quartz vapor lamp negative calcium balances were changed to positive balances in two mature lactating goats and one mature dry goat which had been brought into negative calcium balance or equilibrium on a ration deficient in the antirachitic factor.

Meigs and associates (7) concluded from their own results and a survey of previous balance experiments that the results of such experiments are such as to indicate that the experimental conditions usually interfered with the calcium assimilation. Evidence from balance experiments was also presented to show that the assimilation of phosphorus and probably calcium was favored by the addition of disodium phosphate to the grain ration and feeding the grain and hay of the ration on alternate days. Marked increases in the milk yield in the Beltsville herd were noted when this procedure was practiced (8).

Miller and associates (9) have reported an experiment in which the addition of bone meal to a ration including red clover hay, permitted a storage of calcium and phosphorus.

A further report by Miller and associates (10) dealt with results obtained after the same animals had been maintained under successful lactation for ten months and were still farrow and producing about 20 pounds of milk daily. A storage of calcium and phosphorus resulted on the basal ration which included clover hay, corn silage and grain. Supplements of bone meal and kale separately increased the storage of calcium while with bone meal, phosphorus storage was increased and with kale phosphorus storage was decreased.

Monroe (11) has presented data showing that cows on high protein rations stored calcium while those on lower protein rations lost this element. Combinations of clover and timothy hays were fed, the proportion of clover to timothy being higher in the high protein rations. Beet pulp, carrying a liberal supply of calcium was a constituent of the rations. Some of the results indicated that the narrow rations used would permit a greater phosphorus retention than the wide rations.

In further work Monroe and Perkins (12) have shown that on the same rations cows which had previously had pasture were better able to assimilate calcium than cows which had been continually on dry feed while the reverse was true in the case of phosphorus. The effect of feeding distilled water as compared to that ordinarily offered the herd was determined, there being apparently slight difference in the results.

For the past two years a study of nitrogen balances with dairy cattle has been in progress at this station[2] and in conjunction with this work it was thought worth while to determine the balances of calcium and phosphorus in as much as the roughages and rations used permitted interesting studies of different phases of the problems concerned in the assimilation of calcium and phosphorus. The results of this study are recorded in this paper.

<div align="center">EXPERIMENTAL</div>

<div align="center">*The trials*</div>

The experiments reported in this paper consist of four trials in which the balances of intake against outgo of calcium and

[2] The results of these studies will be published in the near future by L. A. Maynard, W. E. Krauss and the author.

phosphorus were determined. Trials 1 and 2 were conducted in December, 1923, and January, 1924, and trials 3 and 4 in November and December, 1924. In trials 1 and 4 clover hay was the roughage fed and in trials 2 and 3 timothy hay was used. In 1923 the clover hay trial preceded and in 1924 followed the timothy hay trial.

Each trial consisted of three periods: A transition period in which the cows were accustomed to the experimental quarters and fed the roughage characteristic of the trial, but only an approximate grain mixture. There followed a preliminary period in which they received accurately weighed out amounts of the feeds and then a collection period when the excreta was collected by attendants. The transition periods were seven to ten days long, the preliminary periods four days to a week long, and the collection periods in trials 1 and 2, twelve days long, and in trials 3 and 4, fourteen days long.

A point of interest that may affect the results obtained is that the cows were exposed to direct sunlight periodically throughout the experiment. The actual preliminary feeding and collection of excreta was conducted in a north room of a basement barn where there was no exposure to sunlight, but during the transition and preliminary periods the cows were turned out in a courtyard for approximately two hours daily when the weather permitted, and during the collection period they were exercised at least every other day in this courtyard or in the vicinity of the barn. These exercise periods were from twenty to thirty minutes long, the cows being accompanied by attendants equipped for collection of any voided excreta. Whether or not this procedure materially affected the calcium assimilation is a question, but it is thought well to point out that the cows were exposed to some direct sunlight and that some calcium assimilation may have resulted from it. When weather conditions permitted the cows were removed from the stable every other day for the purpose of weighing them.

The cows

The animals used were mature pure bred Holstein cows. The stages of the lactation and gestation periods covered are given in

table 1. Animals 1 and 4 were the same. All of the cows were good feeders with the exception of no. 2 in trial 2, when for two days she refused a considerable amount of feed.

Previous to starting the experiment in 1923 the cows were in good condition, having been on a good grain ration containing 1 per cent each of bone meal and calcium carbonate and having received an excellent quality of mixed hay but rather poor corn silage that contained but few ears.

TABLE 1

Stages of lactation and gestation periods covered and average daily milk yields

TRIAL NUMBER	COW NUMBER	DISTINGUISH- ING FEATURE OF RATION	PERIOD OF LACTATION	PERIOD OF GESTATION	AVERAGE DAILY MILK YIELD	AVERAGE WEIGHT OF COWS
			day	*day*	*pounds*	*pounds*
1	1		101–112	3–14	48.66	1,359
	2	Clover	238–249	Not bred	39.45	1,187
	3	Hay	88– 99	4–15	54.92	1,451
2	1		132–143	34–45	46.44	1,372
	2	Timothy	269–280	13–24	35.51	1,167
	3	Hay	119–130	35–46	53.66	1,447
3	4		74– 87	Not bred	51.31	1,369
	5	Timothy	63– 76	Not bred	48.05	1,281
	6	Hay	188–201	Not bred	37.65	1,301
4	4		99–112	6–19	49.00	1,349
	5	Clover	88–101	6–19	46.25	1,247
	6	Hay	213–226	1–14	34.91	1,279

In 1924 before being placed on the experiment the cows had been receiving a good grain ration in excess of their requirements. They had been getting 8 pounds daily of a mixture of clover and timothy hays and 60 pounds of poor quality corn silage. They had been receiving no minerals.

There is no reason to believe that in either year any of the animals were in a bad state of mineral or vitamin depletion, having not been long off pasture and having since received fair quality rations previous to starting the experiments. It can be said that the previous nutritional history of the cows was probably better than that of cows subjected to average practical

conditions, although they had not been receiving the best quality of hay.

THE FEEDS AND RATIONS

The feeds used in the experiments were such as are commonly used in winter feeding. The ingredient composition of the various grain mixtures is given in table 2. The calcium and phosphorus contents of the feeds is given in table 3. The clover

TABLE 2

Ingredients of grain mixtures, average daily rations consumed and average daily milk produced

| TRIAL | COW | COMPOSITION OF GRAIN MIXTURE | | | | TOTAL GRAIN | TIMOTHY HAY | CLOVER HAY | SILAGE | SALT | MILK | WATER |
		Corn meal	Ground oats	Wheat bran	Oil meal							
		per cent	per cent	per cent	per cent	grams	grams	grams	grams	grams	grams	grams
1	1	59.67	20.17	13.44	6.72	8,358		5,444	18,144	35	22,073	
	2	45.19	27.41	18.27	9.13	6,276		5,226	18,144	35	17,895	
	3	54.65	22.67	15.12	7.56	10,134		5,225	17,517	35	24,910	
2	1	35.29	32.36	21.57	10.78	8,970	5,444		18,144	45	21,067	
	2	25.19	37.40	24.94	12.47	6,794	4,536		12,096	45	16,107	
	3	26.79	36.61	24.40	12.20	10,722	5,444		18,050	45	24,341	
3	4	33.42	33.29	22.19	11.10	8,934	5,460		18,144	60	23,274	48,318
	5	35.22	32.39	21.59	10.80	7,146	5,460		18,144	60	21,796	40,170
	6	40.79	29.61	19.73	9.87	7,244	4,527		17,085	60	17,079	39,334
4	4	36.89	63.81			8,544		5,460	18,144	60	22,228	46,893
	5	61.20	38.80			7,224		4,540	18,144	60	20,977	37,412
	6	65.47	34.53			6,438		4,540	16,689	60	15,833	40,442

and timothy hays used in trials 1 and 2 were judged as Federal grade number 2 and those used in trials 3 and 4 as number 1. The clover hay particularly in trial 4 was of excellent quality. No particular care was taken in the curing process, however, none of the clover was excessively exposed to the elements in curing.

In no case was the silage of excellent quality. It was for the most part corn silage containing sunflowers and very few corn

ears. At the beginning of trials 2 and 4 new silos were opened and for several feedings the silage was not of good quality, it being necessary to exercise care in the feeding in order to keep the cows on feed.

The composition of the grain mixture to be used was ascertained by determining the probable milk yield of the cows by means of the transition period when they were fed a basal grain mixture together with silage and the roughage characteristic of the trial.

In most cases the milk production was actually overestimated so that the cows received rations slightly in excess of their expected requirements based on their performance during the transition period.

TABLE 3

Calcium and phosphorus content of feeds as weighed and fed

	TRIAL 1		TRIAL 2		TRIAL 3		TRIAL 4	
	Ca	P	Ca	P	Ca	P	Ca	P
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Silage.....................	0.1590	0.0657	0.1573	0.0923	0.0983	0.0537	0.0789	0.0409
Clover hay................	1.1123	0.2208					1.1275	0.2790
Timothy hay..............			0.3778	0.2238	0.3131	0.1692		
Grain, cow 1..............	0.0925	0.5087	0.1347	0.7285				
Grain, cow 2..............	0.1073	0.6041	0.1513	0.6440				
Grain, cow 3..............	0.0952	0.5751	0.1362	0.8615				
Grain, cow 4..............					0.1206	0.7252	0.1054	0.3241
Grain, cow 5..............					0.1138	0.6600	0.0811	0.3528
Grain, cow 6..............					0.1190	0.6643	0.0635	0.3182

The rations were made up to supply each cow with the upper limit of total digestible nutrients specified by the Morrison standard, but in order to satisfy conditions desired for the nitrogen balance study the amount of protein fed was necessarily lower than that specified. The protein intake was calculated to be the maintenance requirement plus digestible protein equal to 120 per cent of the protein in the milk produced.

In making up the rations it was desired to feed the same amount of silage and hay in all cases but to vary the composition of the grain mixture to adjust the protein intake to the desired level. Therefore with clover hay a low protein grain mixture was fed and vice versa with timothy hay. The high protein content

TABLE 4

Average daily balances of calcium and phosphorus

		cow 1		cow 2		cow 3	
		Ca	P	Ca	P	Ca	P
		grams	*grams*	*grams*	*grams*	*grams*	*grams*
Trial 1	Silage............	28.849	1.192	28.849	1.192	27.852	1.151
	Clover hay........	60.554	12.020	58.129	11.539	58.118	11.537
	Grain............	7.731	42.517	6.734	37.913	9.648	53.281
	Total............	97.134	55.729	93.712	50.644	95.618	70.969
	Milk.............	24.854	19.071	20.293	15.247	23.067	22.270
	Urine............	0.624	0.211	0.900	0.282	0.542	0.226
	Feces............	69.443	41.357	73.354	41.596	74.394	42.517
	Total............	94.921	60.639	94.547	57.125	98.003	65.013
	Balance...........	+2.213	−4.910	−0.835	−6.481	−2.385	+5.956
Trial 2	Silage............	28,541	1.675	19.027	1.116	28.393	1.666
	Timothy hay......	20.567	12.184	17.137	10.152	20.567	12.184
	Grain............	13.572	57.767	9.152	49.494	14.603	92.370
	Total............	62.680	71.626	45.316	60.762	63.563	106.220
	Milk.............	25.154	21.509	19.908	15.382	26.215	24.536
	Urine............	1.130	0.211	1.357	0.419	0.394	0.254
	Feces............	46.202	54.121	37.030	47.658	50.569	70.654
	Total............	72.486	75.841	58.295	63.459	77.178	95.444
	Balance...........	−9.806	−4.215	−12.979	−2.697	−13.615	+10.776

		cow 4		cow 5		cow 6	
Trial 3	Silage............	17.836	0.974	17.836	0.974	16.795	0.917
	Timothy hay......	17.095	9.238	17.095	9.238	14.174	7.660
	Grain............	10.774	64.789	8.132	47.163	8.620	48.121
	Water............	4.300		3.575		3.501	
	Total............	50.005	75.001	46.638	57.375	43.090	56.698
	Milk.............	26.974	23.786	22.254	22.232	19.675	17.660
	Urine............	0.958	0.237	1.818	0.198	1.676	0.258
	Feces............	32.786	57.275	35.092	46.933	27.174	47.178
	Total............	60.718	81.298	59.164	69.363	48.525	65.096
	Balance...........	−10.713	−6.297	−12.526	−11.988	−5.435	−8.398
Trial 4	Silage............	14.316	0.742	14.316	0.742	13.168	0.683
	Clover hay........	61.562	15.233	51.189	12.667	51.189	12.667

TABLE 4—*Continued*

		cow 4		cow 5		cow 6	
		Ca	P	Ca	P	Ca	P
		grams	*grams*	*grams*	*grams*	*grams*	*grams*
Trial 4	Grain.........	9.005	27.691	5.859	25.486	4.088	20.486
	Water.........	4.174		3.330		3.599	
	Total.........	89.057	43.666	74.694	38.895	72.044	33.836
	Milk..........	25.540	19.516	21.271	19.425	17.749	15.453
	Urine.........	1.056	0.288	1.142	0.230	2.359	0.234
	Feces.........	67.436	32.863	60.872	25.480	58.280	28.813
	Total.........	94.032	52.667	83.285	45.135	78.388	44.500
	Balance.......	−4.975	−9.001	−8.591	−6.240	−6.344	−10.664

of the clover hay used in trial 4 required that the oil meal and wheat bran be omitted from the grain mixture. This ration was therefore comparatively high in calcium and low in phosphorus as can be seen from table 3.

The grain of the ration was fed three times daily at milking time, the hay and silage being fed at night and morning. Very little feed was refused by any of the animals. However, any residue was accounted for and after being dried out in a steam oven was fed back to the cow if possible.

During trials 1 and 2 the cows received distilled water and in trials 3 and 4 tap water such as ordinarily fed to the herd was used. All water taken by the cows was weighed.

The methods of analysis used were the official methods of the Association of Official Agricultural Chemists with the exception that after ashing and dissolving in dilute hydrochloric acid, calcium was determined by the McCrudden method (13) modified to use the potassium permanganate titration (14).

DISCUSSION

Calcium balances

The balances of calcium and phosphorus are given in table 4. All of the calcium balances are negative except one in which a slight storage of this element took place with cow 1 in trial 1 on

clover hay. In general smaller negative balances were obtained when clover instead of timothy hay was fed as the roughage. These results are in agreement with the findings of other investigators. The only exception occurs in the case of cow 6 which lost less than one gram more calcium in trial 4 than in trial 3. In general probably due to advancement in the lactation period the losses of calcium by this animal were low. In addition she suffered some disturbance due to breeding on the morning that trial 4 was started and had difficulty in consuming her allotment of silage for several days.

As has been pointed out, the silage in trials 3 and 4 was of poor quality and not as good as that used the previous year. Consequently the calcium intake was considerably lower in the last two trials. What effect the quality of the silage would have from the standpoint of both its content of calcium and the anti-rachitic factor is questionable. From this work and also the results of Forbes and Hart such a consideration does not seem to be important although it may have a small effect on calcium assimilation.

A comparison of the calcium balances obtained in trials 1 and 4 may indicate the possibility of decreased calcium assimilation in trial 4 due to the low phosphorus content of the ration used in that trial. Such an adjustment of the rations was made necessary by the nitrogen balance studies due to the high protein content of the clover hay that was used.

No difference is noted of the effect of feeding tap water in trials 3 and 4 instead of distilled water as in the first two trials except a possibly higher outgo of calcium in the urine in some cases. This is in general in accord with a recent report by Monroe and Perkins (12). The calcium content of the tap water was low but the intake of this element was accounted for. Only a trace of phosphorus could be detected in the tap water.

The previous nutritional history of the cows each year may have influenced the results obtained. This factor may have been responsible for the relatively low losses and for one case of storage in trial 1 as compared to the greater losses experienced in trial 4 on clover hay. Any residual effect of the previous feeding of

minerals as mentioned before would be a factor that might have
tended to produce the results obtained.

As to the possible influence of sunlight in these experiments
little can be said. No record was kept of the actual number of
hours of exposure but at the most it could not have exceeded
thirty hours in any of the combined preliminary, transition and
collection periods. From a survey of the sunlight records of the
local weather bureau it can be assumed that any possible influence
of sunlight was fairly uniform in all of the trials. Since in these
experiments from this standpoint the treatment approached that
used in general practice, under similar weather conditions it could
be said that the effect of sunlight was not sufficient to counter-
balance the deficiency in the rations of the factor affecting cal-
cium assimilation. Further work should determine what would
be the influence of sunlight under summer conditions. From
work with swine by Maynard and co-workers (15) little differ-
ence was noted between winter and summer sunlight conditions
in the prevention of stiffness in swine. Just what may constitute
an adequate amount of sunlight to induce calcium assimilation
in dairy cattle should be determined.

The question of the accompanying protein metabolism on
mineral assimilation is one which has not been investigated
thoroughly and may be considered in the interpretation of the
results obtained in this work. Monroe (11) has noted negative
calcium balances on wide rations and positive balances on narrow
rations, the narrow rations containing a higher percentage of
freshly cured clover hay. Numerous investigators have demon-
strated the superiority for mineral assimilation of certain rough-
ages over others which are known to contain less protein which
is thought to be of inferior quality. With swine, certain protein
supplements seem to be superior to others in their effect on
mineral asssimilation. Maynard and co-workers (16) working
with swine have noted the beneficial effects of fish meal on mineral
assimilation where a constant plane of protein intake was main-
tained. In many mineral studies the ingredient of the ration
which is being studied carries not only an increased supply of
minerals and perhaps the factor aiding their assimilation but also

Team

Jerseys

1. Iowa.. 1069
    Jersey trophy
2. Ohio.. 1039
3. Illinois... 1015
4. West Virginia.. 1013
5. Kentucky.. 993
6. Michigan.. 992
7. Oklahoma... 989
8. Virginia... 983
9. S. Dakota... 976
10. Texas... 974

Team

All Breeds

1. Iowa.. 4016
    Trophy—N. D. A.
    Trophy—Hoards Dairyman
2. South Dakota.. 3927
    Wyandotte cup by J. B. Ford Company
3. Cornell... 3841
4. Illinois... 3820
5. Ohio... 3800
6. Kansas... 3754
7. Kentucky.. 3726
8. Minnesota... 3710
9. Texas.. 3695
10. West Virginia... 3671
11. Ontario... 3662
12. Oklahoma... 3651
13. North Dakota... 3596
14. Missouri.. 3585
15. Nebraska.. 3581
16. Wisconsin... 3569
17. Tennessee... 3562
18. Massachusetts... 3551
19. Michigan.. 3529
20. Purdue.. 3514

NEWLY ELECTED OFFICERS OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The result of the mail ballot taken in December, 1925, shows the election of the following officers of the American Dairy Science Association for 1926.

President, O. E. Reed..........................East Lansing, Michigan
Vice-President, H. F. Judkins..................Amherst, Massachusetts
Editor, J. H. Frandsen..............................Lincoln, Nebraska
Secy-Treas., G. C. White..........................Storrs, Connecticut
Rep. to National Research Council,
        L. A. Rogers, Bureau of Dairying, Washington, D. C.

more protein of a better quality. It may be logical to attribute the beneficial effects on mineral nutrition to the minerals added or their aided assimilation, however, though it may be only a coincidence, the fact remains that increased mineral assimilation has been accompanied by either or both an increase in the amount of or an improvement in the quality of protein.

In this work a better assimilation of calcium occurred under conditions where protein of supposedly superior quality was used while a constant plane of intake was maintained. In the work of Monroe the plane of protein intake was raised by the substitution of protein of superior quality. It would seem that the quality of protein may be an important factor in the interpretation of the results of studies of mineral metabolism and as such it is offered in this work. A report of the nitrogen balance studies carried on in connection with this work will throw more light on the accompanying protein metabolism.

Phosphorus balances

The phosphorus balances are all negative except in the case of cow 3 in trials 1 and 2. This animal was the highest producer and the heaviest cow used in the experiments and as such required more of a higher protein grain mixture in combination with a fixed amount of silage and hay. From the phosphorus balances resulting is indicated an increased assimilation of phosphorus corresponding with a higher intake than that of the other cows. Accompanying this condition were negative calcium balances. Such independence of calcium and phosphorus assimilation has been noted at various times by several workers. It is further brought out in an examination of the remaining balances obtained in this work. The explanation for such a storage as seen in the case of cow 3 is difficult. There is perhaps little possibility of a longstanding condition of this kind but again it may be, in this case, the result of a previous condition where this cow was not receiving phosphorus enough to cover her requirements. More flexibility in the mobilization of phosphorus is possible than in the case of calcium, due to the higher content of phosphorus in the softer tissues of the body in addition to that contained in the bones.

In addition, in three other cases, the phosphorus balances although negative indicate increased utilization of phosphorus when its content in the grain mixture was increased. As in the case of cow 3 it might be possible for a cow to be in a negative calcium balance and phosphorus equilibrium at the same time. Such a possibility has been pointed out by Meigs (8).

Hart and co-workers (2) have observed that the quality of the hay not only affects the assimilation of calcium but also that of phosphorus. With an increased assimilation of calcium in such a case we might also expect an increased assimilation of phosphorus. The balances determined in this work are not generally in agreement with such a statement but may be similar to those

TABLE 5

Calcium and phosphorus content of milk

COW NUMBER	TRIAL 1		TRIAL 2	
	Ca	P	Ca	P
	per cent	*per cent*	*per cent*	*per cent*
1	0.1126	0.0864	0.1194	0.1021
2	0.1134	0.0852	0.1236	0.0955
3	0.0926	0.0894	0.1077	0.1008
	TRIAL 3		TRIAL 4	
4	0.1159	0.1022	0.1149	0.0878
5	0.1021	0.1020	0.1014	0.0926
6	0.1152	0.1034	0.1121	0.0976

obtained later by Hart (5) when due to a generous provision of wheat bran in the ration positive phosphorus balances accompanied calcium losses on green grass. It is therefore of practical interest that the phosphorus demands of dairy cattle can be provided for by adequate grain mixtures although it is questionable whether or not this is actually achieved in practice.

The calcium and phosphorus content of the milk is given in table 5. An inspection of these data indicates that the concentration of these elements in milk is fairly constant. A comparison of the composition of the milk from the same cow in the different trials shows in all cases a slightly higher calcium content when the cows received timothy hay instead of clover hay.

Greater differences are noted in the same direction in all instances in the case of phosphorus. To be sure, the differences observed are small but they are fairly uniform and if significant they bear out the previously mentioned contention of an increased assimilation of phosphorus in response to the increased supply of this element in trials 2 and 3 over that of trials 1 and 2.

There is a popular belief that the mineral content of cows' milk is altered in response to an increased mineral assimilation. From table 5 it can be seen that any variation that may have occurred in this case is very small and was probably the result of an increased mobilization of phosphorus rather than of calcium.

SUMMARY

1. The results of twelve determinations of the balances of intake against outgo of calcium and phosphorus with liberally milking dairy cows are given.

2. A better assimilation of calcium resulted when clover hay instead of timothy was fed as part of a ration which included corn silage and a grain mixture.

3. A better assimilation of calcium occurred under conditions where protein of supposedly higher quality was fed through the medium of clover hay.

4. Some independence between calcium and phosphorus assimilation was observed.

5. Better assimilation of phosphorus occurred when more ample provision was made for this element in the grain mixture.

6. There was but little variation in the calcium and phosphorus content of the milk under the conditions studied. The percentage of phosphorus in the milk seemed to increase slightly in response to an increased supply in the feed.

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ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The twentieth annual meeting of the American Dairy Science Association was held in connection with the National Dairy Exposition at Indianapolis, Indiana, on October 12 and 13, 1925.

The first general session which convened at the Hotel Severin at 9:00 a.m. October 12 was called to order by President O. E. Reed who spoke briefly of the work of the Association. The minutes of the 1924 meeting were read and approved. The report of the secretary-treasurer, J. B. Fitch, was read and adopted.

Divisional meetings were reported on by:

A. A. Borland for eastern division.

C. A. Hutton for southern division.

F. W. Atkeson read a report submitted by H. A. Bendixen for the western division.

C. H. Eckles reported on the Union of Biological Societies. He outlined the plans of the Union and stated that Rockefeller Foundation has $350,000 to be spent in ten years for the publication of abstracts. This would be available January 1, 1926.

C. H. Eckles also reported as representative to the National Research Council.

At the request of W. E. Skinner, the President appointed a committee to study the Dairy Exposition and to make suggestions for improvement. The following committee was appointed: W. A. Stocking, F. W. Atkeson, H. H. Kildee, and E. M. Harmon.

The President also appointed the following members of the National Research Council Advisory Committee:

A. A. Borland and R. S. Breed their terms expiring July 31, 1926.

H. A. Ruehe and W. M. Regan whose terms expire July 31, 1927.

O. F. Hunziker and L. A. Rogers whose terms expire July 31, 1928.

The representative to the National Research Council is to act as chairman.

H. P. Davis spoke briefly on the advisability of appointing a committee to study the courses in dairying offered at the different institutions with the idea of unifying and making more standard the courses offered. No action was taken on this suggestion.

J. H. Frandsen spoke regarding the JOURNAL OF DAIRY SCIENCE, outlining future plans for that publication.

C. H. Eckles suggested that the allowance of $300 for the editor of the Journal of Dairy Science was inadequate and warranted an increase more in keeping with the experience and duties of that position. President Reed appointed a committee composed of Eckles, Larson and Stocking to study and make recommendations.

The following nominations for 1926 were reported by A. A. Borland, chairman of the nominating committee:

> For President: O. E. Reed, Michigan; J. M. Fuller, New Hampshire.
>
> Vice-President: H. F. Judkins, Massachusetts; A. C. Baer, Oklahoma.
>
> Secretary-Treasurer: G. C. White, Connecticut; H. A. Ruehe, Illinois.
>
> Editor: J. H. Frandsen, Nebraska; A. C. Dahlberg, New York.

The final selection is to be made by mail ballot as provided in the constitution.

Announcements were made concerning the meeting for the next day and concerning the banquet. The meeting adjourned at noon, to convene again October 13, after the sectional meetings.

SECOND SESSION, TUESDAY OCTOBER 13, 1925, 9:00 A.M.

After the meetings of the different sections a general session was called for Tuesday, October 13, 1925. J. A. Gamble reported on the Production Section, H. F. Judkins for the Manufacturing Section, L. W. Morley for the Extension Section and C. E. Wiley for the Advanced Registry Section.

A. A. Borland spoke briefly on the uniform cow testing associa-

tion rules adopted by the Extension Section. It was his idea that they were very much the same as the proposed Ayrshire herd test. Ragsdale, Morley and Baltzer also spoke regarding the uniform rules.

C. H. Eckles submitted the following report from the committee appointed to study the allowance for the Editor of the JOURNAL:

"The committee recommends to the Executive Committee that the sum of $300 be added to the budget for the Editor's office the payment of this additional sum to be contingent upon an equal sum being brought into the hands of the Treasurer as a result of the proposed membership campaign."

It was the idea of the meeting that no increase could be made unless the membership was increased. With this in mind all agreed to help the Secretary in increasing the membership. Mortensen, Borland, Hayden and Stocking spoke regarding the membership of the Association.

The following budget for 1926 was submitted as approved by the Executive Committee:

Editor's allowance	$300
Secretary's salary	100
Secretary's office	100
Three divisions	75
	$575

The following officers were elected in the different sectional meetings:

Section I Production:

C. C. Hayden, Chairman

O. G. Schaefer, Secretary

Section II Manufacturing:

A. C. Baer, Chairman

C. D. Dahle, Secretary

Section III Extension:

A. J. Cramer, Chairman

C. F. Finley, Vice-Chairman

C. R. Gearhart, Secretary

Section IV Advanced Registry:
  C. E. Wylie, Chairman
  W. E. Petersen, Secretary

C. H. Eckles reported for the committee on the revision of the constitution.

The annual banquet was held at the Hotel Severin at 6:30 Tuesday evening, October 13. H. F. Judkins acted as toastmaster and A. J. Glover, editor of Hoard's Dairyman was the principal speaker.

Announcements were made of awards and presentation of prizes in Intercollegiate Judging Contests. Mr. Wm. White presented the awards to winners in Dairy Products and Mr. W. W. Swett, to winners in the Dairy Cattle Contest.

J. B. FITCH,
Secretary.

PRODUCTION SECTION

The Production Section of the American Dairy Science Association was called to order by Professor J. A. Gamble, Chairman. Secretary J. P. LaMaster being absent, Professor A. C. Ragsdale acted as secretary.

The minutes of the last meeting were read and approved.

Professor H. E. Van Norman, representing the National Dairy Association gave an address dealing particularly with the Farmers Judging Contest and placing card. This placing card had been recommended to the National Dairy Association and adopted for use this year by a committee appointed the previous year by this section of the American Dairy Science Association. This committee reported as follows:

Your Committee on Dairy Cattle Placing Card for Farmers Judging Contest submits the following report:

Several informal meetings of the Committee were held with three of the four members present—not always the same ones.

Taking the placing card devised by the Animal Husbandry Department of the University of Wisconsin, and used at the last National Dairy Show, as a starting point, the accompanying placing card was approved by the Committee and recommended to the National Dairy Show for use this year, which recommendation has been accepted.

The card was printed as per copies attached herewith and samples mailed by the National Dairy Association to 77 dairy department professors in 35 states. National Dairy Association offered to supply any desired number at cost. Nine professors secured for trial, copies in varying numbers from a few to several hundred, while Wisconsin printed ten thousand just like this.

Inquiries as to the working out of this score card in practice were directed to the professors who secured additional copies for use. Seven replied unanimously approving the placing card arrangement as submitted herewith. One instructor did raise the question as to whether the final placing should not count more than 50 per cent of the total score, which question the Committee does not attempt to answer, as the 50–50 basis is used in the National Students Judging Contest and other similar contests.

Referring to the key for scoring the contestants, four instructors reported using this key, one without modification and three modifying the values to fit the classes being judged.

One department which has not used the card suggests omitting the key from the card, while Professor Humphrey, who has had the largest experience in the use of this card, would supply a complete key with all possible combinations shown with their values. This would require 24 tables like the one printed on the reverse of this card.

Your Committee recommends that the American Dairy Science Association receive this report, give such publicity to the placing card herewith submitted as circumstances may permit; further, that the Committee continue to collect further data concerning the use of this card and report at the next annual meeting such suggestions for improvements or modifications as circumstances may dictate; and further that the Association authorize the printing of the following phrase on these placing cards:

Placing Card Recommended by the American Dairy Science Association

Report respectfully submitted,
<div align="right">

SPECIAL COMMITTEE ON FARMERS
JUDGING PLACING CARD,
J. C. HUMPHREY
H. H. KILDEE
W. W. SWETT
H. E. VAN NORMAN, *Chairman.*
</div>

The report of this special committee on the Farmers Judging Placing Card was accepted, placed on file and the committee continued.

W. W. Swett, Chairman of the Committee on the Students Dairy Cattle Judging Contest, gave the following report:

The annual meeting of the committee on the Students Dairy Cattle Judging Contests was held at the office of the National Dairy Association, Chicago, Illinois, March 21st, 1925. The meeting was called to order by the Chairman. The following members of the Committee were present: A. A. Borland, L. H. Fairchild, H. H. Kildee, W. W. Swett; and also H. E. Van Norman and S. H. Anderson of the National Dairy Association.

The material presented to the Committee by the chairman consisted of suggestions received relative to methods of improving the conduct of the contest work. These suggestions were read over and carefully considered.

Only one outstanding change in the rules was made at this annual meeting. This change provides that the superintendent shall select a judging committee, composed of two judges, preferably college men or ex-college men of recognized ability on all four breeds, to act with a special breed judge for each breed. It was the sense and understanding of the committee that the special breed judge shall have the qualifications and duties of the associate judge of 1924, that he shall have equal voice with the two other members of the committee in placing the rings of the particular breed on which he is working and that the committee shall decide on one placing for each class by working together rather than independently. This decision was reached after a lengthy discussion by each of the members of the committee which revealed within the committee a very strong sentiment for developing the contest on a basis in which the college view point would predominate.

A short time after the annual spring meeting a circular letter signed by the coaches of 24 different state institutions came to the Chairman unsolicited. This circular letter made a number of specific suggestions and recommendations for changes in the rules governing the contest. The changes made at the meeting were, in all essentials, directly in accord with those written requests from 24 different states.

A number of other minor changes in the wording were necessary to make the rules conform to the one major change outlined. The question of the relative merits of oral and written reasons was discussed thoroughly but no change was made in this regard.

Preliminary arrangements were outlined for the annual banquet of the American Dairy Science Association. The responsibility for this banquet was placed on Professor Borland.

The question of some kind of a score card for simplifying the contest procedure was discussed, but no action was taken.

The rules as amended were unanimously adopted as a whole.

The meeting adjourned at 5:00 p.m.

> A. A. BORLAND,
> L. H. FAIRCHILD,
> J. B. FITCH,
> J. M. FULLER,
> L. W. INGHAM,
> H. H. KILDEE,
> W. M. REGAN,
> W. W. SWETT, *Chairman.*

This report was approved and placed on file.

Dr. Sewell Wright, of the United States Department of Agriculture gave a most excellent address on *Inbreeding and Crossbreeding.*

R. R. Graves, of the United States Department of Agriculture, gave an address on Breeding Dairy Cattle for High Milk Production. This address together with that of Dr. Wright, occasioned some little comment and were much appreciated.

The report of the nominating committee was unanimously approved and C. C. Hayden, Ohio, and O. G. Schafer, Minnesota, were declared elected president and secretary respectively of Section I.

The meeting adjourned at 4:15 p.m.

> A. C. RAGSDALE,
> *Acting Secretary.*

MANUFACTURING SECTION

The Dairy Manufacturers' Section of the American Dairy Science Association was called to order by Chairman, H. F. Judkins on October 12, 1925 at the Hotel Severin at Indianapolis, Ind.

The minutes were read by Secretary N. E. Olson and approved.

Professor Judkins reported for the Committee on Judging

Dairy Products. This report recommended the inclusion of ice cream in the next year's contest.

The report which follows was adopted as read:

Report of Rules Committee for the Students Dairy Products Judging Contest at the National Dairy Show, October 12, 1925

Rule 13 now reads, "Each contestant shall score and criticize ten tubs of butter, ten cheeses, and ten samples of milk, the time allowance being one hour for each product." It is recommended that the following be added, "A five minute rest period shall be allowed at the end of each one hour judging period."

The last part of Rule 16 reads, "The contestants shall have access to these samples, five minutes for each product, just previous to the scoring of the contest samples. To make it clearly understood that the contestants cannot refer to these samples while the judging of the contest samples is in progress the words, "and then only," should be added. It is recommended that the time for examination of these samples be changed to ten minutes.

It is recommended that the rules committee be left with power to decide whether ice cream shall be included in the contest next year with the understanding that they will be guided by the judgment of the coaches who participate in the practice judging tomorrow. If ice cream is included next year it is recommended that the number of samples of each product be changed from ten to seven and the time for judging each product changed from one hour to forty-five minutes.

The first paragraph under judges, page eight, reads, "The superintendent of the contest shall select a committee of two judges to judge each product and shall, as far in advance of the contest as possible, notify each institution as to the personnel of these committees."

It is recommended that the last part of the sentence relative to notifying the colleges as to the personnel of the judges be omitted.

Score cards

In arranging the defects under "bottle and cap" on the milk score card the bottle defects should be arranged separately from the cap defects. It should be made clear just what score is given to the different types of caps. It is recommended that if ice cream is judged next year that on the student's score card the defects be stated as on the score cards for other products, that is, not grouped under a definite range of score as they are now.

The committee is always open to suggestions as to how to secure more prizes and as to what the nature of these prizes should be.

Respectfully submitted,

M. MORTENSEN,
H. W. GREGORY,
G. D. TURNBOW,
W. H. WHITE,
H. F. JUDKINS,
Rules Committee.

NOTE: No further action was taken at the meeting in regard to including ice cream in next year's contest than that stated in committee's report.

Dr. R. S. Breed reported for the Committee on Bacteriological Methods. The report was approved and the committee continued. It was suggested that a sub-committee for bacteriological methods for ice cream be appointed. The suggestion was approved by vote and Chairman Judkins appointed, A. C. Fay, F. W. Fabian, and B. W. Hammer. Motion that this committee be instructed to act with a committee appointed by the National Association of Ice Cream Manufacturers, carried. Professor Judkins appointed himself a committee of one to present the matter to the ice cream men's convention at Detroit.

Dr. Harding suggested a separate score card for pasteurized milk. Professor Fisher moved that the suggestion be referred to the score card committee. Carried.

Mr. R. C. Potts reported for the Committee on Economic Phases of the Dairy Industry. Report approved.

Professor C. L. Roadhouse presented a report on "Judging Dairy Products in Our High Schools." An interesting motion picture of the activities of the dairy department, at Davis, California, was also exhibited.

O. R. Overman, Urbana, Ill., presented a paper on "The Relation of Solids in Milk to Fat and Specific Gravity of the Milk." Ordered placed on file.

R. C. Fisher, Cincinnati, Ohio, reported at length for the Committee on Testing Ice Cream for Fats and Solids. The report was ordered placed on file and the committee continued. It was suggested by A. C. Dahlberg that a central committee on Chemi-

cal Methods of Testing Dairy Products be appointed. Moved by R. C. Fisher that such committee be appointed to be composed of the chairman of the other committees on Testing Dairy Products. Motion carried and the following committee appointed, O. F. Hunziker, Chairman, R. C. Fisher, H. C. Troy, and A. C. Dahlberg.

Dr. Otto Rahn of Kiel, Germany, next delivered an interesting report on the Dairy Physics Department of the Government Dairy School in Kiel.

A number of interesting reports of research work included the following:

"Federal State Butter Inspection as a Means for Quality Improvement." C. W. Fryhofer, St. Paul, Minn.

"Correlation of the Scored Butter and Its Methods of Manufacture on Yeasts and Molds in Butter." E. A. Parfitt, Lafayette, Ind.

"The Effect of Ice Cream Ingredients Upon the Viscosity of the Mix." D. A. Downs, Lincoln, Nebr.

Professor P. M. Brandt delivered a thorough report on "The Operation of College Creameries."

Professor A. C. Baer was called upon to tell of the successful operation of a coöperative creamery in conjunction with the College at Stillwater, Oklahoma. The report was approved.

Professor Roadhouse then suggested that a committee be appointed to present the above report to the National Dairy Organizations such as The National Milk Dealers' Association, The Ice Cream Manufacturers Association, The American Association of Creamery Butter Manufacturers, The National Buttermakers' Association, and the National Cheese Association.

The suggestion was approved and Chairman Judkins appointed the following committee: C. L. Roadhouse, Chairman, O. F. Hunziker and P. M. Brant.

Election of officers of Section II resulted as follows: A. C. Baer, Stillwater, Okla., Chairman; C. D. Dahle, State College, Penn., Secretary.

The meeting adjourned at 6:15 p.m. until 1926.

N. E. OLSON,
Retiring Secretary.

EXTENSION SECTION

The Extension Section this year held one of the most interesting meetings in its history. About 70 members were present from 18 states and the District of Columbia. The committee reports showed that much work had been done by the various committees, and splendid papers were read on subjects of timely interest.

The meeting was called to order by the Chairman, L. W. Morley. Minutes of the last meeting were read by the Secretary, C. A. Hutton, and approved.

Reports of the various committees were called for. Professor J. D. Brew, Chairman of the Dairy Manufacturing Committee, was absent.

Professor E. B. Fitts, Chairman of the Feeding Committee, submitted a written report together with copies for each member of the Extension Section. Professor Fitts emphasized the importance of practical suggestions in bulletins and other printed material, as well as in talks to demonstrators about feeding methods and rations. He referred to a number of useful bulletins which have been issued by various dairy specialists during the year. He also called attention to the value of the feeding school, conducted in the demonstrator's barn, as a useful method of interesting the dairymen in the community in improved rations.

Miss Jessie M. Hoover, Chairman of the Milk Campaign Committee, said she would submit a mimeographed report for members.

Mr. L. A. Higgins, Chairman of the Bull Association Committee, was not present, and the report was read by the Secretary and adopted by the meeting.

An interesting discussion followed and a motion was adopted that the work of the committee be broadened to include dairy sire work in general.

Mr. E. J. Perry, Chairman of the Calf Club Committee, submitted a written report which was read and adopted. The recommendations of this committee were as follows:

1. That high average results per member rather than a large enrollment should be the goal.

2. That membership should first be enlisted from among those responsible families most in need of good dairy animals.

3. That subject matter for use by calf clubs should be prepared by the extension specialists in consultation with the dairy department.

4. That a manual or bulletin containing in simple form the fundamentals of management be used, supplemented with seasonal circular letters.

   Circular letters may well contain questions to be answered by the members from reference bulletins, manuals, etc., the answers to be returned to the club leader or specialist.

5. It is strongly urged that in follow-up work every effort be put forth by club leaders and dairy extension workers to secure yearly records on all milking heifers.

6. That the records of milking animals be summarized, first by the various states and then nationally. These records while privately made should be used in contrast with the United States average production per cow to strengthen the calf club work throughout the country.

Mr. A. B. Baltzer, Chairman of the Cow Testing Association Committee, submitted a lengthy and very interesting written report. A very interesting discussion followed. A motion was carried that the report be adopted and presented to general session of American Dairy Science Association. This report showed that the committee had given a great deal of time and thought to the task of working out a system for uniform methods of cow testing association work throughout the various states. The report covered standard rules and regulations for the operation of testing associations. It was later presented to the general session of the American Dairy Science Association, where it was unanimously adopted.

"Methods of Dairy Extension and Follow-up Work" was the subject of a paper by Mr. E. J. Perry. Mr. Perry brought out some interesting points in regard to new methods of systematic planning of extension work.

Mr. J. B. Parker gave a report on short-cut methods in cow test association work. He emphasized sticking to the regular cow-test association plan wherever possible.

The following officers were elected: Chairman, A. J. Cramer, Wisconsin; Vice-Chairman, C. B. Finley, Iowa; Secretary, C. R. Gearhart, Pennsylvania.

Chairman Morley announced that a new plan was being introduced this year, whereby the members of the Extension Section will be furnished copies of the various papers and committee reports.

Adjournment.

NOTE: Copies of the committee reports and papers have been sent to the members of the Extension Section. Any member failing to receive copies, or desiring additional copies, should communicate with Mr. C. R. Gearhart, Secretary, State College, Pa.

C. A. HUTTON,
Secretary.

ADVANCED REGISTRY SECTION

Meeting of the Advanced Registry Section of the American Dairy Science Association, Monday, October 12, 1925, at the Severin Hotel, Indianapolis, Indiana.

In the absence of Professor G. C. White of Connecticut, the section elected C. E. Wylie of Tennessee to act as Chairman. M. H. Campbell of Illinois was appointed as Acting Secretary. C. E. Wylie, as Secretary, read a report of the minutes of the last meeting at Milwaukee. Mr. Harris of Wisconsin gave the following report for the Investigation Committee.

Conclusions of Investigation Committee

It would seem that sufficient data has been presented to warrant the conclusion that the one-day test, at least under normal conditions, is about as reliable as the one for two days. Three independent studies show very close agreement in results and larger numbers would evidently reduce the variation still further. It is therefore pertinent to consider what, if any, abnormal conditions might affect the reliability of the one-day test to a greater extent than would obtain in a test for a longer period.

Experiments to determine the effects of various feeds indicate that such effect is, generally speaking, not immediate and usually not apparent until the second day. Darnell of Texas is continuing this in-

vestigation, preliminary results being in accord with the above. If this be true, the shorter period is an advantage. There is room for further investigation along this line and also to determine the effect of lengthening or shortening the interval between milkings, changing method of milking and feeding or not feeding cows while being milked.

Professor Colman, Oregon, submitted a report entitled "A Study of the Estimated Versus the Actual Milk Weights as Reported by the Breeders" which is not included in the body of this report only because of lack of space.

<div style="text-align:center">Respectfully submitted,

ROY T. HARRIS, *Chairman*,
Committee on Investigations.</div>

Mr. Harris' report was accepted.

Mr. Musser of the American Guernsey Cattle Club, brought up a question requiring the supervisor not to talk during a test. It was moved and seconded and passed that this be referred back to the Breeds' Relation Committee.

Professor Conklin of the Ayrshire Breeders' Association presented the plan of the Ayrshire herd test. He stated that this plan was presented to the committee for their suggestions and criticisms, in as much as it was the plan of this test to have the Advanced Registry Officials in the several states to supervise the herd test system.

Ayrshire Herd Test Plan

1. The Test shall be available to Ayrshire herds, grade and purebred.

2. When a herd is entered in the Test every cow which has ever freshened or freshens subsequently shall be included without reference to period of lactation.

3. All cows must be identified by tattooing.

4. Cows shall not be milked more than twice per day, except when producing as much as the following quantities: mature cows, 40 pounds, four year olds, 35 pounds; three year olds, 30 pounds, and two year olds, 25 pounds.

5. The Test shall be for a period of twelve months and breeders are urged to enter their herds with the intention of continuing them in the Test year after year.

6. The Test shall be based upon a one day inspection of milk and fat

production each month with such additional surprise tests as may be deemed necessary for authentication. It is strongly recommended that daily milk weights be kept by the owner to indicate the exact milk production. Lacking daily records, the month's credit for milk shall be computed from the day's production.

7. A maximum of 20 cows may be included in one day's test when none are milked more than twice per day. In no case shall the test include more than forty milkings per day.

8. No minimum qualifying production requirements for herd averages shall be set. In calculating herd averages the production of all cows which have ever freshened and are owned in the herd eight months or more of the year shall be counted.

9. Supervision and authentication of Herd Tests in the several States shall be under the direction of the Agricultural Colleges. Cow testing association supervisors may conduct the tests provided the requirements of the Ayrshire Breeders' Association and the supervising College are met, it being understood in such an instance that the supervisor while engaged in the work of the Herd Test is considered the employee and representative of the college and responsible to it. Payment for his work shall be made by the College, fees being paid the College by the herd owner. Where no cow testing association is available supervision may be made by the men employed by the College in official and semi-official testing or by such other qualified agencies as may be agreed upon by the Ayrshire Breeders' Association and the College.

10. To enable owners of herds entered in this test to secure the extra authentication and publicity of Roll of Honor records on outstanding individuals it is provided that semi-official tests may be conducted in connection with the Herd Test, provided the requirements of this test and of Article III of the By-Laws are met.

11. A herd test book shall be furnished by the Ayrshire Breeders' Association to each owner whose herd completes a year's test. This shall be arranged to provide a separate page for each cow on which is given for each month the production of milk during the day of the test, the fat percentage, amount of milk and fat for the month, the value of the product, the amount and cost of feed, and the profit or loss. In the back there shall be space for a summary of the year's work of each cow tested and for the seal of the Ayrshire Breeders' Association.

12. Upon receipt of information that a breeder desires to enter his herd in this test the Ayrshire Breeders' Association shall mail a blank

which the applicant shall fill out and forward to the Ayrshire Breeders' Association together with fees to cover the Association's charge for the first month. Provision shall be made on this blank for an application for test which shall incorporate a certification that the data submitted on the herd is accurate and that the applicant will endeavor to see that the test data collected accurately measures the production of the herd. The following information shall be given for all cows in the herd which have ever freshened, Registry number if recorded, tattoo marks, age, date of last calving, service dates, etc.

Upon acceptance of an application for a herd test the Association shall make arrangements for the beginning of the monthly supervisions. Monthly records shall be sent to the Ayrshire Breeders' Association and entered there in the herd test book. Carbon copies shall be left on the farm. At the end of the year the book shall be summarized by the Ayrshire Breeders' Association and sent to the herd owner as a permanent record of his herd's performance.

13. Individual herd test certificates. Certificates of individual records of meritorious cows in the herd test shall be furnished when such cows produce as much as the following quantities of milk and fat specified during the 305 days of the test and drop living calves within 400 days of the previous freshening and such records are accepted by the Ayrshire Breeders' Association:

	MINIMUM REQUIREMENTS	
	Milk	Fat
	pounds	*pounds*
Mature..	7,500	300
Senior Four...................................	7,000	280
Junior Four...................................	6,600	264
Senior Three..................................	6,150	246
Junior Three..................................	5,700	228
Senior Two....................................	5,250	210
Junior Two....................................	4,800	192

14. Sires with four daughters out of four different dams, qualifying for Herd Test Certificates, shall be designated as Herd Test Sires, and each assigned a number.

15. Fees. Charges for tests shall be borne by owner, including surprise tests and any special tests for authentication which may be deemed necessary by the Ayrshire Breeders' Association or the Agricultural College.

A fee of twenty cents per month per cow in milk shall be charged by the Ayrshire Breeders' Association for computing the records, publication of accepted records, blanks, herd books, etc. This fee shall be payable in advance.

The first part of the committee meeting was devoted entirely and at some length to a discussion of this plan of testing. Those present t ought that the principles of this test were of far reaching importance to the conduct of Advanced Registry Testing and therefore this subject was the most important subject to be considered at this meeting. This plan of testing includes the testing of all cows in Ayrshire herds both grade and purebred. It provides for a one-day test, as many as twenty cows on test in a day, one or more cows milked at a time, cow testing association or Advanced Registry Testers and authentication of the test reports by the colleges. Conklin, Reed and Campbell expressed the idea that there was a tendency for this kind of testing to increase. Regan, Fitch and Wing thought that this idea was a compromise between Advanced Registry Testing and Cow Testing Association Records. Everyone seemed to agree that the rules for Advanced Registry Testing should not be materially lowered as they were the outgrowth of many years of constant revision. At the same time everyone seemed to think that the herd test plan was a good thing to encourage.

It was moved and passed that the Breeds' Relation Committee approve the conducting of the herd test by the Supervisor of Advanced Registry of the various states so long as the rules of the American Dairy Science Association governing official testing are followed. A motion was passed favoring the appointment of one man from the Breeds' Relation Committee to consider with a man appointed from the Investigation Committee, the herd test plan.

Regan of California presented a plan for uniform guarantee for payment of Advanced Registry Testing. This report which follows was adopted:

Report of Sub-Committee on Uniform Guarantee in Payment Testing Accounts

Resolved, That we of the Testing Section of the American Dairy Science Association, feeling keenly the need for a more definite and uniform practice as regards guarantee of testing accounts by the various cattle clubs, do recommend the following as the preferred practice in handling such accounts.

1. That the college concerned render bill to the owner for tests conducted as promptly as possible after the work is completed.

2. If bills rendered for as much as two months work be not paid by the time the statement for the third month is ready, such statement should be made out listing items in full with dates of service rendered and presented to the cattle club concerned.

3. If such statement covering three months testing is not paid by the owner within 15 days following the date of rendering such bill to the cattle club the college notify the owner that he is delinquent and that testing in his herd is and has been suspended until such time as his account shall be paid.

4. If the delinquent breeder fails to make payment within 15 days following the date when he is declared delinquent, the college is to render final detailed statement to the cattle club (no further tests being conducted in the meantime) and the cattle club to make payment to the college within 30 days or as soon as items can be properly verified.

Be it further resolved that the Chairman of the Breeds' Relation Committee be instructed to transmit these resolutions to the proper official of the respective breed associations requesting their adoption by them and their incorporation in the breed handbooks and literature on official testing.

R. T. HARRIS,
W. M. REGAN.

It was brought to the attention of this committee that a rule should be made to prohibit the talking of test supervisors or in otherwise interfering with a cow where he is supervising the testing. There was also a discussion in regard to a rule governing the adulteration of sulfuric acid. No motion was passed concerning these two points but it was thought best to properly instruct the supervisor on these two subjects.

A motion was passed that Advanced Registry Section favor

either a one-day test with preliminary milking or a two-day test with preliminary milking in the conduct of Advanced Registry Testing.

Signed: C. E. WYLIE, *Chairman*,
M. H. CAMPBELL,
J. B. FITCH,
O. E. REED,
J. R. DICE.

Following the Report of the Breeds' Relation Committee Professor Regan asked for a discussion regarding the Ayrshire herd test plan. Professor Conklin of the Ayrshire Breeders' Association presented this plan to the meeting. There was considerable discussion on many phases of this plan. It was moved, seconded and passed to amend the Breeds' Relation Report as follows:

That the supervision of a herd test plan follow in a general way, the rules as proposed by the Ayrshire herd test plan and that differences between this and the Advanced Registry rules be adjusted by a sub-committee of the Breeds' Relation Committee, such committee to work with the Ayrshire Breeders' Association and other associations during the year and to come before this section with certain recommendations next year. The report of the committee as amended was approved unanimously.

Professor Conklin of the Ayrshire Breeders' Association proposed a College Students' Essay Contest on the Economical Value of Production Tests. It was moved and passed that this plan be referred to the general session and that a man be appointed to represent the American Dairy Science Association on the Contest Committee.

In the absence of W. E. Peterson of Minnesota, Mr. Harris of Wisconsin read a paper prepared by Mr. Peterson on "The Adulteration of Sulfuric Acid Used in Testing for Advanced Registry." This paper was summarized as follows:

1. Most fats and oils added to the sulfuric acid will increase the test of milk when such acid is used. All such fats and oils float on top of the acid and cause it to become very dark and sirupy, but do not affect its activity.

2. Many fat solvents when added to sulfuric acid will increase the test of milk where such acid is used. All float on top of the acid, but many do not affect the appearance of the acid nor its activity. Duplicate samples of milk where such acid is used will fail to check within permissible limits.

3. Saturated solutions of butterfat in gasoline, benzine and xylol will not materially affect the appearance of cold acid and will remain in solution with the acid for an hour or more after becoming thoroughly mixed. Successive samples of acid drawn will produce identical results; all samples checking in duplicate within the permissible limits of variation.

4. The presence of any substance in the sulfuric acid that will increase the test of milk when such acid is used in testing can be detected by making a "blank" test of the acid.

Mr. Davidson of Illinois presented a paper on "The Methods of Detecting the Leaving of Milk in the Cow's Udder." Mr. Johnson of Connecticut Agricultural College, gave a report of "The Study of the Padding of Milk Weights."

On ballot C. E. Wylie was elected Chairman, W. E. Peterson Secretary of Section IV, for 1926.

The meeting adjourned.

<div style="text-align: right">C. ELMER WYLIE, <i>Secretary,</i>
<i>per M. H. Campbell.</i></div>

REPORT OF THE NINTH STUDENTS NATIONAL CONTEST IN JUDGING DAIRY PRODUCTS

Held at Indianapolis, Indiana, October 12, 1925

The personnel of the official judging committees was as follows:

For Butter:
> O. S. Hagen, Bureau of Agricultural Economics, U. S. Department of Agriculture.
> C. L. Pier, Bureau of Agricultural Economics, U. S. Department of Agriculture.

For Cheese:
> C. W. Fryhofer, Bureau of Agricultural Economics, U. S. Department of Agriculture.

For Milk:

> R. J. Posson, Bureau of Dairying, U. S. Department of Agriculture.

> C. J. Babcock, Bureau of Dairying, U. S. Department of Agriculture.

The trophies offered in this contest and the winners of these trophies this year are as follows:

> National Dairy Exposition cup for team highest in all products Iowa State College.

> J. G. Cherry cup for team highest in butter.   South Dakota State College.

> Hoard's Dairyman cup for team highest in cheese. Iowa State College.

> J. B. Ford cup for team highest in milk.   West Virginia University.

> National Federation of Milk Producers cup to institution having highest individual in milk.   W. B. S. Woodward, Ohio State University.

> Gold medal for highest individual in judging all products, given by National Dairy Exposition.   W. W. Richman, Pennsylvania State College.

> Silver medal for second highest individual in judging all products, given by National Dairy Exposition.   Snowden R. Clary, Iowa State College.

> Bronze medal for third highest individual in judging all products, given by National Dairy Exposition.   Walter L. Olson, Iowa State College.

> Gold medal for highest individual in butter.   American Creamery Butter Mfgrs. Association.   Walter L. Olson, Iowa State College.

> Silver medal for second highest individual in butter.   National Dairy Exposition.   Emery Bartle, South Dakota State College.

> Bronze medal for third highest individual in butter.   National Dairy Exposition.   Daniel Jacobsen, South Dakota State College.

> Gold medal for highest individual in cheese.   National Cheese Association.   Snowden R. Clary, Iowa State College.

> Silver medal for second highest individual in cheese.   National Dairy Exposition.   Ralph P. Wilson, Iowa State College.

Bronze medal for third highest in cheese.   National Dairy Exposition.   W. W. Richman, Pennsylvania State College.

Gold medal for highest individual in milk.   International Milk Dealers Association.   W. B. S. Woodward, Ohio State University.

Silver medal for second highest individual in milk.   National Dairy Exposition.   Geo. N. Gray, Oregon Agricultural College.

Bronze medal for third highest individual in milk.   National Dairy Exposition.   L. L. Lough, West Virginia University.

REPORT OF THE SEVENTEENTH STUDENTS DAIRY CATTLE JUDGING CONTEST INDIANAPOLIS, INDIANA, OCTOBER 10, 1925

Twenty-four teams were entered in this contest, twenty of which are listed in the table showing the standing in all breeds.

Individuals

Ayrshires (no prize)

Name	State	Score
1. W. N. Wehr	Ohio	378
2. W. S. Bishopp	New York	373
3. B. J. Griffin	Kentucky	371
4. Albert I. Mann	Massachusetts	361
5. H. H. Hannam	Ontario	359
6. M. L. Paul	Texas	348
7. O. L. Ryall	North Dakota	348
8. Ross Miller	Nebraska	341
9. T. Martell	North Dakota	339
10. F. J. Arnold	Iowa	335

Individuals

Guernseys

1. D. M. Seath	Iowa	375
2. F. J. Arnold	Iowa	375
3. C. N. Dotson	Oklahoma	375
4. O. F. Garrett	Illinois	360
5. C. L. Blakeslee	Minnesota	360
6. R. Goley	Oklahoma	360
7. R. K. Mitchell	New York	355
8. H. L. Peterson	Texas	355
9. C. W. Thole	Kansas	355
10. G. Bowman	Virginia	350

Teams

Ayrshires

1. Kentucky.. 1001
   Ayrshire trophy—Ayrshire Breeders Association
2. Ontario... 995
3. Ohio.. 986
4. Texas... 984
5. Massachusetts... 979
6. North Dakota... 967
7. New York (Cornell)... 951
8. South Dakota... 325
9. D. R. Willia... 325
10. O. F. Garrett.....been found to be......Illinois 325

Individuals

Jerseys

1. J. E. Craig...Wisconsin   380
   AJCC Scholarship $400
2. D. M. Seath..Iowa   375
3. L. F. Steiner..Ohio   375
4. R. C. Ferguson.......................................Iowa   369
5. T. M. Miller...Indiana   367
6. G. H. Faulconer......................................Kansas   365
7. O. F. Garrett..Illinois   363
8. R. E. Horwood..Michigan   362
9. M. L. Paul...Texas   361
10. A. E. Skidmore......................................Oregon   360

Individuals

All breeds

1. E. Bartle..S. Dakota   1380
   Gold Medal—National Dairy Association
   Cane—Dairy Farmer
2. D. M. Seath..Iowa   1365
   Silver Medal—National Dairy Association
   Cane—Dairy Farmer
3. O. F. Garrett..Illinois   1361
   Bronze Medal—National Dairy Association
   Cane—Dairy Farmer
4. C. L. Blakeslee......................................Minnesota   1343
5. G. H. Faulconer......................................Kansas   1341
6. R. C. Ferguson.......................................Iowa   1338
7. W. S. Bishopp..New York   1327
8. R. H. Smith..S. Dakota   1317
9. F. J. Arnold...Iowa   1313
10. W. N. Wehr..Ohio   1304

dividuals

olsteins

. C. L. Blakeslee.....................................Minnesota   370
>   Holstein-Friesian Scholarship $400
:. E. Bartle..S. Dakota   365
:. R. K. Mitchell..New York   335
:. D. M. Seath...Iowa   335
:. D. E. Borror...Oregon   329
' A. I. Mann...Massachusetts   325
    9. Kladd..New York   325   951
    10. Iowa...Tennessee   947
                                                        Mass.............. 944

Teams

Guernseys

1. Iowa... 1090
    Guernsey trophy—American Guernsey Cattle Club
    Guernsey placque—American Guernsey Cattle Club
                [R. C. Ferguson
                | D. M. Seath
    Book Ends { F. J. Arnold
                | C. T. Peterson (Alt.)
                [Prof. F. Ely (Coach)
2. Oklahoma.. 1045
3. Kansas.. 1025
4. S. Dakota... 1015
5. Minnesota... 990
6. Cornell... 980
7. Illinois.. 980
8. Ontario... 975
9. Nebraska.. 940
10. Missouri... 935

Team

Holstein

1. S. Dakota... 985
    Holstein trophy
2. Cornell... 970
3. Illinois.. 946
4. Oregon.. 931
5. Iowa.. 913
6. Purdue.. 909
7. Minnesota... 885
8. North Dakota.. 873
9. Wisconsin... 871
10. Massachusetts.. 870

THE RÔLE OF VITAMIN A IN THE NUTRITION OF CALVES*

I. R. JONES, C. H. ECKLES AND L. S. PALMER

University of Minnesota, St. Paul, Minnesota

INTRODUCTION

Vitamin A has been found to be an indispensable factor in the diet of a number of species of animals. The most characteristic symptoms of a deficiency resulting from its absence, determined especially in case of small laboratory animals, are failure to grow, xerophthalmia, respiratory trouble, intestinal disturbances, death and, with mature animals, failure of normal reproduction and rearing of young. The question seems pertinent as to how far the results obtained with laboratory animals are applicable to domestic, ruminating animals, concerning which no systematic study has been found in the literature.

It will be the purpose of this paper to set forth the results of an investigation to determine if ruminants, as represented by calves, require a supply of vitamin A for normal growth and well-being. Although the problem was attacked largely from the scientific aspect, the economic consideration was kept in mind. With approximately 4,500,000 calves of the dairy breeds alone raised yearly any investigation dealing with their normal nutrition may be of tremendous practical significance.

HISTORICAL REVIEW

The discovery of vitamin A was based upon the fact that it is present in butterfat, but not in lard (1) (2). The vitamin was

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designated as "fat-soluble A" by McCollum and Kennedy (3) because of its solubility in fats and fat solvents. However, the vitamin is not necessarily associated with fats as, for example, when found in green plants. Also recent investigations (4) (5) have shown that there are two fat-soluble vitamins, one specific in the prevention of xerophthalmia, the other in the prevention of rickets. The anti-xerophthalmic factor is now usually designated as vitamin A and the anti-rachitic factor as vitamin D.

The chemical nature of vitamin A is unknown. The vitamin is quite stable towards heat in the absence of air and oxidizing agents. The body has a considerable capacity for storage of vitamin A, especially in the liver (6).

Some of the symptoms of vitamin A deficiency in the rat are failure to grow (1) (2), xerophthalmia (7) (8), infections of the respiratory tract (9) (10), intestinal atrophy and thrombopenia (11), and osteoporosis (12). Cessation of growth, keratomalacia and an epizootic were observed in guinea pigs on a vitamin A deficient diet (12) (13). Rabbits ceased to grow and developed xerophthalmia on such a diet (14). Chickens likewise ceased to grow (15) and developed xerophthalmia (16) (17) on a diet lacking vitamin A.

Kittens fed on a vitamin A deficient diet ceased growing, developed diarrhea and showed intestinal atrophy (18). Dogs on such a diet developed xerophthalmia (19). Pigs would not grow in the absence of vitamin A (20).

In case of the human, especially children, xerophthalmia and keratomalacia are very common when the vitamin A content of the diet is inadequate (21) (22). Failure to grow and respiratory infections are other symptoms.

As yet there is nothing definite in the literature as to the mode of action of vitamin A. Cramer (11) believes that it acts as a hormone stimulating food absorption. Keith and Mitchell (23) think that it is "involved in the metabolism incident to the increased activity of the voluntary muscles." Sherman (24) suggests that vitamin A may be related to tissue building, it possibly being an important constituent of tissue.

Factors involved in the problem

The investigator conducting experiments with large animals involving deficient rations is confronted with many difficulties that the worker with small laboratory animals does not meet. This is especially the case in vitamin deficiency studies. The main difficulty is the formulation of suitable rations. The preparation of a ration of purified food stuffs for large animal experiments is a practical impossibility due to the large amount of food consumed, the length of the growth cycle and the cost of such a ration. The problem then is to select natural foodstuffs that are lacking or deficient in the factor studied or which can be made so in some practical manner. In the case of herbivora the ration must be selected from the limited supply of feeds that this group will consume. Also the ration of herbivora is composed largely of roughage, and it is exceedingly difficult to grow or maintain an animal of this group on a ration composed exclusively of concentrates. Natural roughages consist largely of the leafy part of plants, and as vitamin A is synthesized in the leaves of plants it is a difficult matter to find a natural roughage deficient in the vitamin.

It is a recognized fact that vitamin A is stored in the bodies of the young to a greater or less extent, depending on the diet of the mother. This being the case, it is essential that the ration of the dam be known for the period while the young were *in utero* if an attempt is to be made to measure the requirement of the young for this factor. This is especially difficult in the case of herbivora because of the lack of definite information concerning the vitamin content of many of the ordinary feeds.

PLAN OF EXPERIMENT

The plan was to place a small number of calves on a ration selected as suitable for animals of this kind and thought to be adequate except in vitamin A. At the same time tests of each ingredient of the ration for vitamin A were to be started with rats. With the information at hand from these preliminary tests it was

planned to formulate definite plans for the rations to be used in the more extensive experiments to follow.

Calves were selected so far as possible from dams whose rations were known for the periods while the calves were *in utero* in order to indicate their possible vitamin A storage. The calves were obtained as soon after birth as possible so that their ration was regulated and accurate records of feed consumption and growth kept from an early age. The calves were to be continued on experiment until at least one year of age, unless they developed symptoms of a deficiency before that time, in which case they would either be continued on the same ration until other symptoms developed, or would be given a supply of vitamin A in an attempt to bring them back to normal.

ANIMALS USED

Six experimental animals (E-38, E-47, E-57, E-63, E-64 and E-66) and three control animals (E-46, E-56, and E-65) were used in the investigation. E-38, E-46 and E-47 were placed on experiment before definite information was at hand concerning the vitamin A content of the various ingredients of the ration. All the animals used were either grade or purebred Holsteins.

RATIONS USED

Milk was used as one of the basal constituents of the ration because of the well recognized fact that milk contains the dietary constituents necessary for growing mammals. The calves were given untreated whole milk for a limited period and then changed to whole or skimmilk treated in a manner thought to destroy any vitamin A present as a ration deficient in this factor was desired. Later the calves were given skimmilk remade from oxidized skimmilk powder or, if they would consume it, skimmilk powder in the dry form. The method used in treating the liquid milk and the skimmilk powder was based on the fact that oxidation destroys vitamin A. After some experimenting with calf E-38 the following procedure was adopted for treating the milk: (a) 10 cc. of hydrogen peroxide were added for each pound of milk;

(*b*) the milk was heated to 60°C. in a water bath and held at that temperature for one hour; (*c*) liquid oxygen from a storage drum was allowed to bubble through the milk at the rate of about 60 cubic feet per hour for each forty pounds of milk. Skimmilk treated in the above manner was tested with rats to determine its
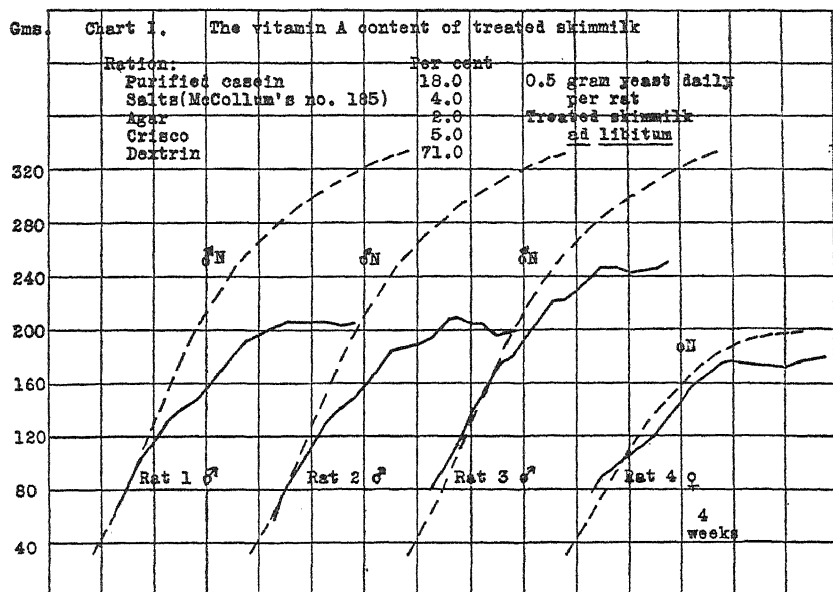
Fig. 1

Rats 1 and 3 were 34 days, and rats 2 and 4, 33 days of age when placed on the test ration. The consumption of treated skimmilk varied from 15 to 35 cc. daily per rat, with an average consumption throughout the test of about 25 cc. The mothers of these rats received a diet very rich in vitamin A as it included 5 per cent alfalfa, 3 per cent cod liver oil and whole milk *ad libitum*. This undoubtedly accounts for the long period of growth before retardation set in. The results indicate a deficiency of vitamin A in the treated skimmilk.

vitamin A content with the results shown in figure 1. The results indicate a deficiency of vitamin A in the skimmilk.

The skimmilk powder used was manufactured by the spray process. The powder was exposed to air and light on the assumption that any vitamin A present would be destroyed by oxidation. The procedure was to spread the powder in shallow layers in pans and boxes placed outside in the sun during the summer
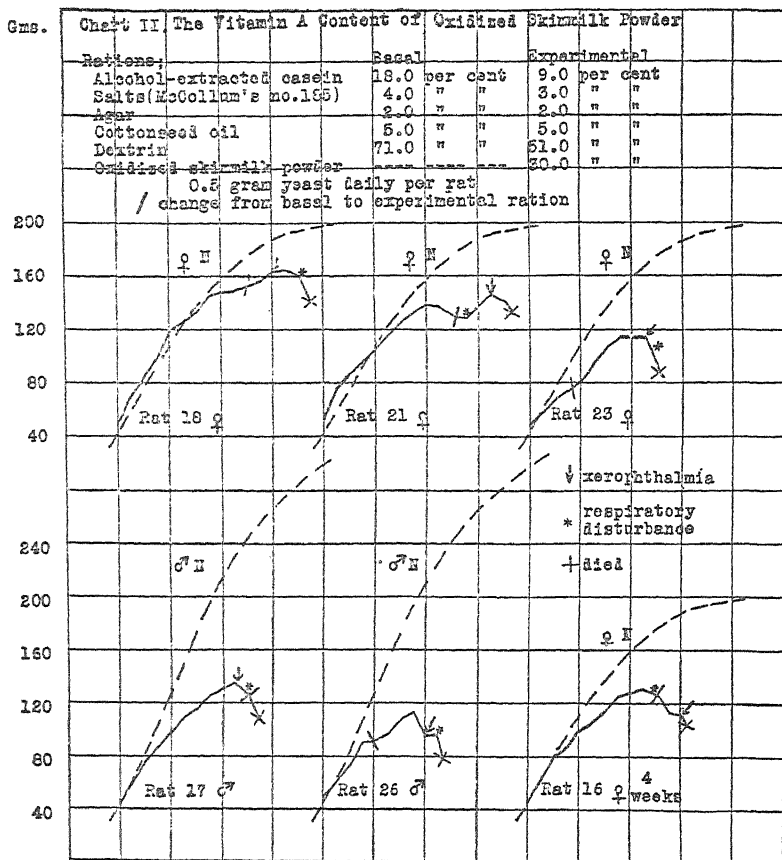
FIG. 2

The rats used in the oxidized skimmilk powder test represented animals from three different litters, the other rats being used in the white corn, wheat straw and dried beet pulp tests. Rats 16, 17, 18 and 21 were placed on the basal ration at 26 days and rats 23 and 26 at 30 days of age. Only in case of rat 17 did xerophthalmia develop before the change to the experimental ration. However, all the other rats later developed xerophthalmia and died of vitamin A deficiency showing that the oxidized skimmilk powder was free of vitamin A. The average weekly food consumption per rat was 46 grams and 44 grams for the basal and experimental rations respectively.

months and inside on a warm cement floor during the winter months. The powder was frequently raked over and was exposed until it changed from its original yellowish to a characteris-

tic white color at which time it also became tallowy in flavor. The oxidation process required five or six days exposure in direct sunlight and three to four weeks in indirect light. The oxidized powder was tested on rats with the results shown in figure 2. The rats in this case were placed on a basal diet lacking in vitamin
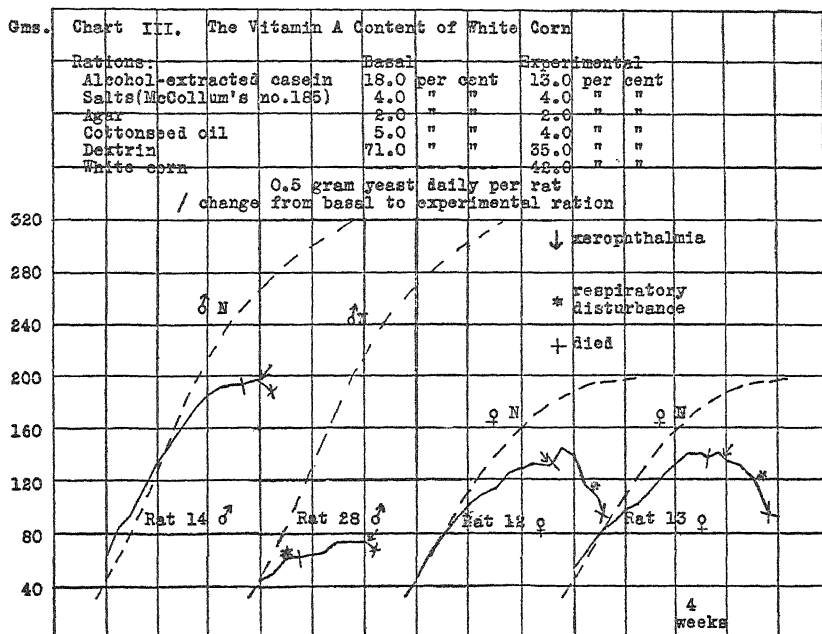
FIG. 3

The four rats used in this test represented three litters. Rat 12 was given the basal ration at the age of 26 days and rats 13, 14 and 28 at 30 days. The change to the experimental ration did not bring about resumption of growth or heal xerophthalmia in the one rat affected. On the other hand, the three other rats developed xerophthalmia before death. The average weekly food intake per rat was about 55 grams of both the basal and experimental rations. The results show that white corn (Rustler White variety) is lacking in vitamin A.

A until they developed symptoms of vitamin A deficiency, at which time the skimmilk powder was substituted for 30 per cent of the diet. The results with six rats show conclusively that the oxidized skimmilk powder was practically free of vitamin A for all of the rats died of vitamin A deficiency.

White corn (Rustler White variety) was used as a source of
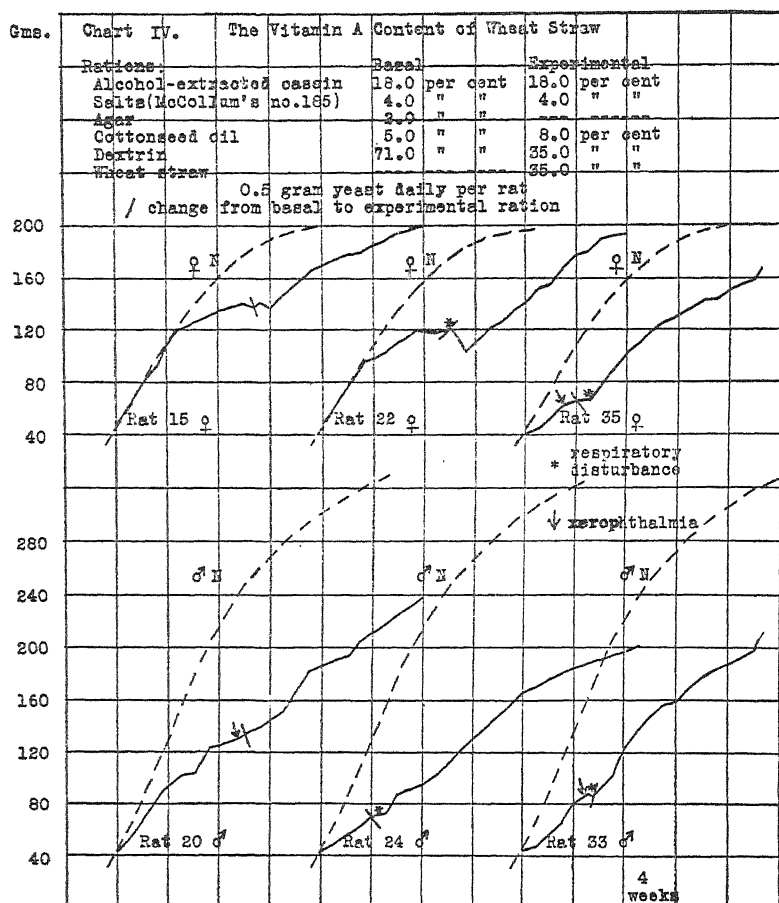
FIG. 4

The 6 rats used in the above test represented four litters. Rats 15, 20 and 22 were placed on the basal ration at 26 days of age, rat 24 at 30 days and rats 33 and 35 at 29 days. The chart shows that a change to the wheat straw ration caused a resumption of growth in case of every rat. The xerophthalmia was healed in five to seven days and the respiratory infection warded off in the rats affected. The average weekly food consumption per rat was 45 grams and 85 grams of the basal and experimental rations, respectively.

energy and vitamin B. Harshaw (25) found that 25 per cent of white corn as the sole source of vitamin B enables rats to make normal growth. Steenbock and Boutwell (26) were unable to demonstrate any vitamin A in white corn. The white corn used

in this experiment was tested with rats to determine its vitamin A content. The results with four rats, as seen in figure 3, show conclusively that white corn (Rustler White variety) is practically free of vitamin A.

Wheat straw was given as a roughage to calves E-38, E-46 and E-47. As pointed out previously, herbivora require roughage for normal growth and behavior and natural roughages probably contain more or less vitamin A. The straws of the cereal grains were thought to offer the best chance of success in finding a roughage deficient in vitamin A; therefore the selection of wheat straw. The vitamin A content of the wheat straw, determined by rat feeding tests, was strikingly different than anticipated. As seen in figure 4, the results with six rats show conclusively that wheat straw is a good source of vitamin A. Dried beet pulp was next selected as a roughage possibly lacking in vitamin A factor. Rat tests were made with the results set forth in figure 7. These show that dried beet pulp is decidedly lacking in vitamin A.

Lemon juice was given to calves E-38, E-46 and E-47 as a source of vitamin C. Osborne and Mendel (27) found that lemon juice contains only a trace, if any, of fat-soluble vitamins. Lemon juice was omitted from the ration of calves other than the above mentioned animals because Thurston, Eckles and Palmer (30) have found that calves do not require vitamin C for normal growth and well-being.

Mineral supplements of calcium carbonate or of calcium carbonate and calcium phosphate were added to the rations of the calves to give an approximate by weight ratio of 1.5 of calcium to 1 of phosphorus which ratio McCollum and co-workers (28) regard as about the optimum for rats.

Cod liver oil was included in the ration of the control animals as a source of vitamin A. In the case of calf E-38 oxidized cod liver oil was given as an anti-rachitic agent during the winter months. The oxidation of the cod liver oil was carried out somewhat in the manner reported by Steenbock and Nelson (5). The other calves were amply supplied with sunshine during most of the experimental periods.

The calves were stabled in individual pens. Shavings were used for bedding. All experimental calves were turned out in a yard together on all days unless the weather was very bad. The yard is located so that it is exposed to the sun the greater part of the day. White corn and wheat straw or dried beet pulp were given to the calves *ad libitum* while the other ingredients of the ration were fed in definite amounts. Salt was always available and water was amply supplied.

Accurate records of feed consumption were kept. Growth was ascertained by weighing every ten days with a three-day weighing period every thirty days and by measuring the height at withers every thirty days. Daily observations of the calves were made and any abnormal symptoms noted. Photographs were taken at various intervals to portray the development of the animals.

RESULTS OF EXPERIMENTS ON CALVES

Experimental calf E-38. Male; dam received pasture seven months and dry feedstuffs two months of gestation period. E-38 was kept on experiment for 402 days without any abnormal symptoms resulting. The growth by weight compared to the Eckles (29) normal Holstein curve is shown in figure 5. As shown in figure 6, the liver of E-38 was found by rat tests to contain considerable vitamin A.

Control calf E-46. Female; dam received dry feedstuffs throughout gestation period. As E-46 was a control animal cod liver oil was fed at the rate of 20 cc. of the oil daily beginning with the 12th day. E-46 was kept on experiment 327 days during which time no abnormal symptoms developed. Her growth curve is shown in figure 5.

Experimental calf E-47. Male; dam received dry feedstuffs throughout gestation period. The ration of E-47 was identical to that of E-46 except that no cod liver oil was given. The growth curve of E-47 is shown in figure 5. This shows that his rate of growth declined rapidly when about 120 days old until at 190 days he was actually losing in weight. At this time he was emaciated in appearance, lacking in vitality and scouring. At 225 days he reacted to the tuberculin test. He continued to decline so that at 230 days he was at the point of death, so cod liver oil was given as a source of vitamin A. Improvement in vitality and in the condition of the fecal matter was soon noticeable

and while he declined further in weight this was soon checked and when taken off experiment at 345 days of age he was gaining at a supernormal rate for that age and was fairly thrifty in appearance. Post-mortem examination showed calcified glandular tuberculosis, chronic peritonitis and chronic pleurisy. Indications were that the calf had been in a more severe state of infection and was gradually recovering.
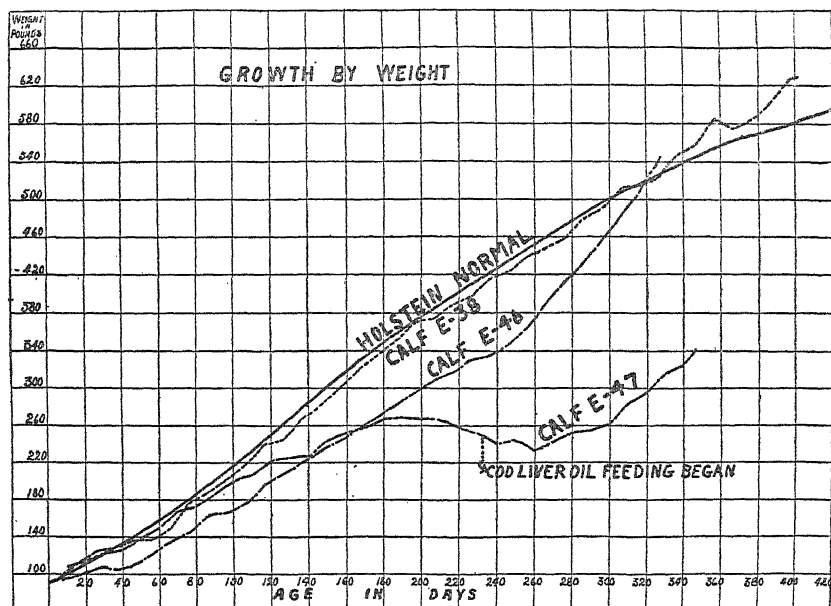
FIG. 5. GROWTH BY WEIGHT OF EXPERIMENTAL CALVES E-38 AND E-47 AND CONTROL CALF E-46

This chart shows that calves E-38 and E-46 were above normal when taken off experiment. Neither of these calves developed symptoms of deficiency throughout the experiment. On the other hand calf E-47 stopped growing at about 180 days and was soon declining in weight. He was near death when cod liver oil feeding began. He slowly recovered and when taken off experiment at the age of 345 days he was growing at a supernormal rate.

Control calf E-56. Male; identical twin with E-57; among other feeds his dam received pasture throughout practically the entire gestation period. Twenty cubic centimeter of cod liver oil was added to the ration daily beginning with the 33rd day. E-56 showed no abnormal symptoms whatever during the 268 days on experiment. His growth curve compared to the normal and to that of E-57 is shown in figure 8.

Experimental calf E-57. Identical twin with control calf E-56.

E-57 received an identical ration to E-56 except that no cod liver oil was given. E-57 was unthrifty and scouring at 75 days of age. The
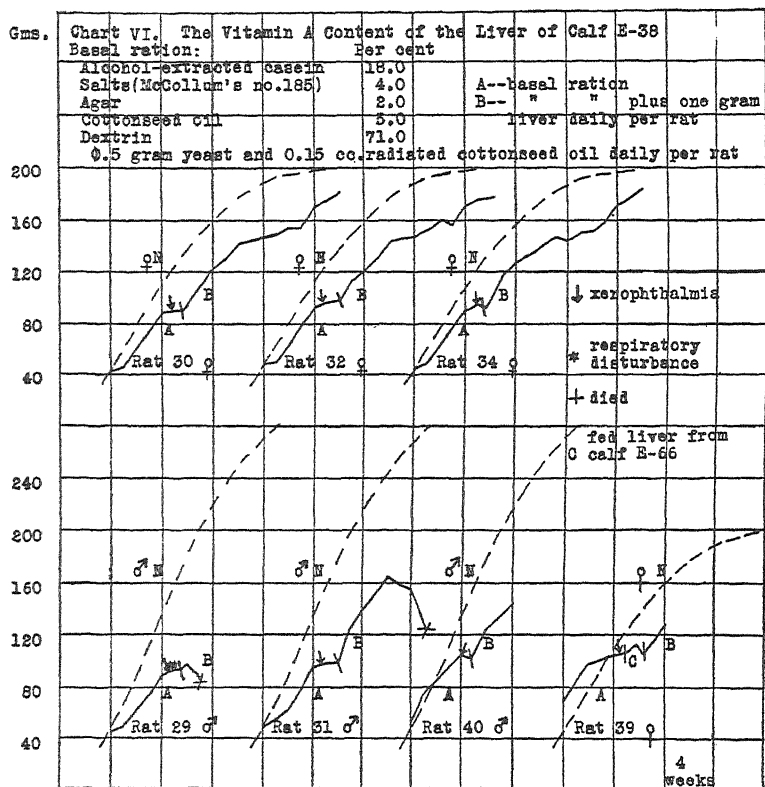
FIG. 6

Rats 29, 30, 31, 32 and 34, littermates, were placed on the basal ration at the age of 29 days. Rats 39 and 40 were started at 31 days of age. One gram of the liver of calf E-38 was given to each rat daily when growth had practically ceased and xerophthalmia had developed. In the case of every rat the xerophthalmia was healed and, except in case of rat 29, growth was resumed. Rat 29 had a bad respiratory infection when the liver supplement was given and did not recover. Rat 31 showed no symptoms of vitamin A deficiency at death. Death was probably due to the fact that a portion of liver that had been kept in a small ice box refrigerator had been fed for a few days. The results show that the liver of calf E-38 contained vitamin A.

scours continued irregularly until at 107 days he was scouring profusely and his eyes were running considerably. His growth curve is given in

figure 8.   This shows that he started to decline in rate of growth at about 75 days and continued to decline until his death at the age of 123 days.   The results show that E-57 was unable to grow and ward off infections in the absence of vitamin A whereas his twin brother on the control ration grew normally.

Experimental calf E-63.   Male; his dam received a ration of alfalfa hay, corn silage and a grain mixture for the first three months of the
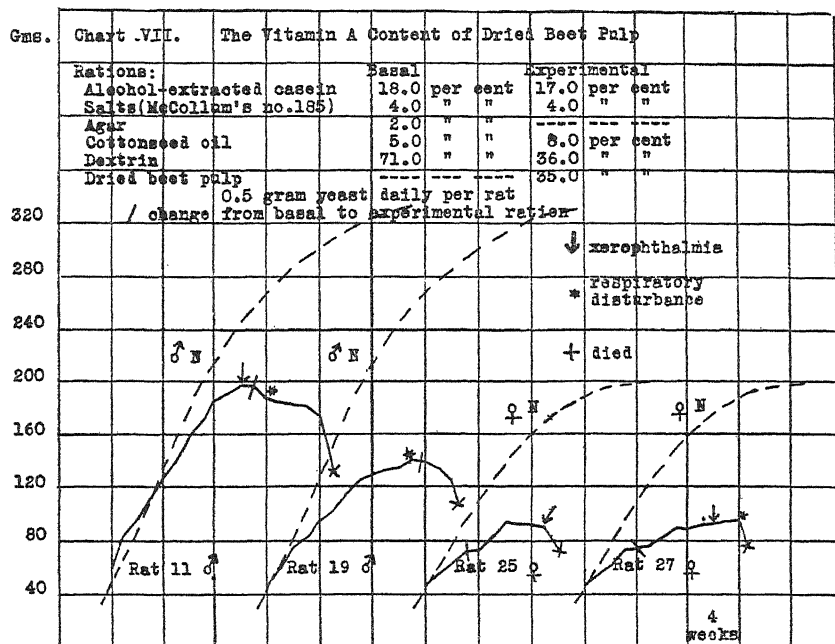
FIG. 7

The four rats used in the dried beet pulp test represented three litters. Rat 11 developed xerophthalmia before the change to the experimental ration and rats 25 and 27 after the change. Rat 19 did not recover from a respiratory disturbance after the change of ration. The average weekly food consumption was 57 grams and 45 grams of the basal and experimental rations, respectively. The results show quite conclusively that dried beet pulp is lacking in vitamin A.

gestation period, then pasture and corn silage for two months, pasture only for the following two months and alfalfa hay, corn silage, and a grain mixture for the two months before calving.   Calf E-63 was un-thrifty and scouring at two months of age, but as figure 8 shows, he grew at a subnormal rate until about 90 days of age when he was very weak, lacking in vitality and scouring.   At the age of 100 days he

went down and would not attempt to arise or stand when put on his feet. His condition did not improve so cod liver oil was added to his ration as a source of vitamin A on the 102nd day. Improvement in the condition of scours, appetite and vitality was almost immediate.
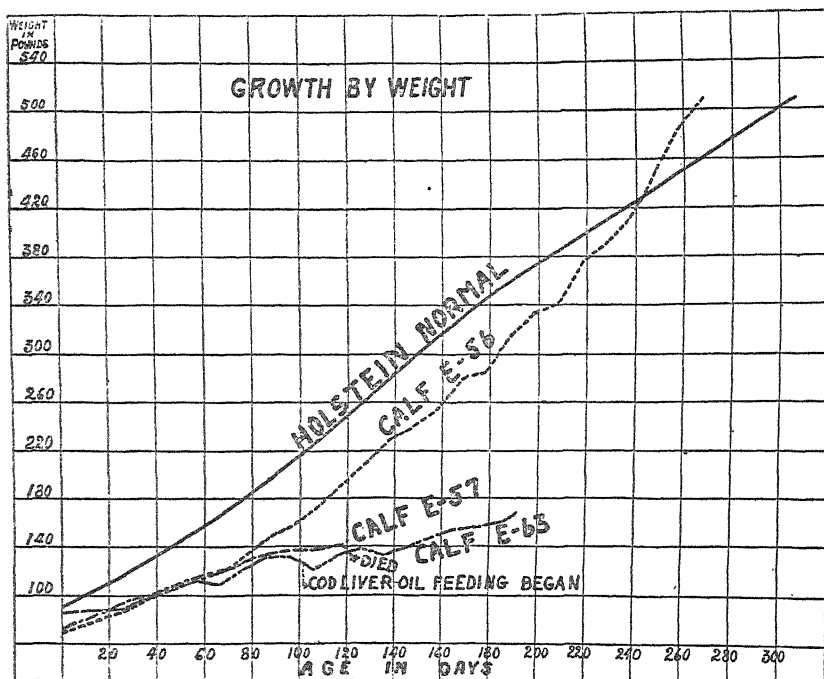
FIG. 8. GROWTH BY WEIGHT OF CONTROL CALF E-56 AND EXPERIMENTAL CALVES E-57 AND E-63

Calves E-56 and E-57 were identical twins. E-56, being a control animal was given 20 cc. of cod liver oil daily in addition to the experimental ration. This calf completed an experimental period of 268 days without showing any abnormal symptoms whatever. On the other hand E-57 died at the age of 130 days showing symptoms of vitamin A deficiency. Calf E-63 received an identical ration to that of calf E-57. At the age of 100 days E-63 was unable to arise and showed symptoms of vitamin A deficiency. Cod liver oil was given as a source of vitamin A and as a result he showed gradual improvement.

He attempted to arise in about ten days, but it was not until the 153rd day that he could get up on all four feet and even then he could not entirely straighten up on his rear legs. As seen in figure 8, E-63 slowly gained in weight after cod liver oil feeding began. The condition of his rear legs did not improve when placed on a normal calf ration.

Postmortem examination showed that the femur of one leg had been broken in two and had partly grown together after the ends had slipped by each other.

‖ *Experimental calf E-64.* Male; one of a pair of ordinary twins, E-65, a female being the other; his dam received the same ration as the dam
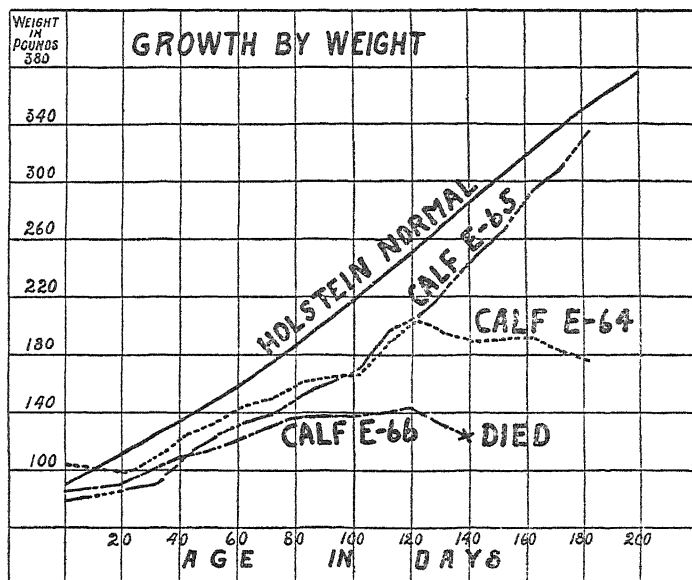
FIG. 9. GROWTH BY WEIGHT OF CONTROL CALF E-65 AND EXPERIMENTAL CALVES E-64 AND E-66.

Calves E-64 and E-66 received a vitamin A deficient ration of oxidized skimmilk powder, white corn meal, dried beet pulp, calcium carbonate, and calcium phosphate. Calf E-65, a twin with calf E-64, received the same ration with the addition of cod liver oil as a source of vitamin A. E-65 showed no abnormal symptoms whatever throughout the experimental period of 183 days. On the other hand calf E-66 died at the age of 139 days and calf E-64 was killed when near death at the age of 183 days. Both of these calves developed xerophthalmia, became blind, ran at the nose, were affected with a chronic condition of scours, and, on post-mortem examination, showed pneumonia, oedema and intestinal atrophy. The results conclusively show that calves require vitamin A in the ration.

of E-63. The growth by weight curve of E-64 is shown in figure 9. At 100 days he did not show the thrifty appearance of his sister on the control ration. At about the 120th day his eyes began to water and strings of mucous were running from his nose. From the 125th day on he declined in weight. At the age of 150 days a puslike discharge
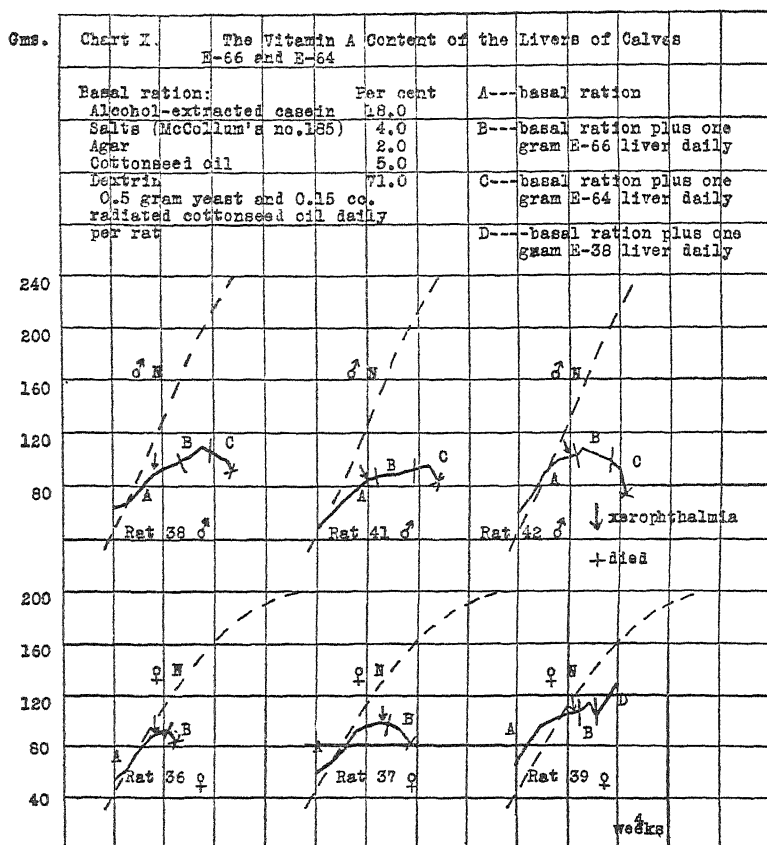
FIG. 10

The six rats used in the above tests were littermates. Rat 40, given E-38 liver, was also from this litter. All of the rats had developed xerophthalmia and practically ceased growing before the liver of calf E-66 was given. This calf died of vitamin A deficiency. In no case did 1 gram of this calf's liver heal xerophthalmia or cause a permanent resumption of growth. Two of the rats died. Of the other four, three were changed to liver from calf E-64, which calf was killed when near death due to vitamin A deficiency, and the fourth, rat 39, was given liver from calf E-38. The rats given E-64 liver did not recover and soon died, whereas rat 39 recovered from xerophthalmia in five days and resumed growth thus corroborating the results shown in figure 6. The results show that vitamin A was practically absent in the livers of calves E-66 and E-64.

was running from his eyes and nose, his feces were very thin and his breathing was interspersed with coughs. Plate 1 shows the marked

contrast in E-64 and his sister at the age of 177 days. At 180 days he was completely blind and xerosis of the eyes was setting in. Long strings of whitish mucous were hanging from his nose. As the calf was in such a miserable state that he would undoubtedly die soon, he was killed on the 183rd day. Growth of the skeleton was totally inhibited during the last twenty days of experiment. Post-mortem examination showed pneumonia, edema of the kidneys, atrophy of the intestines, necrosis of the rumen and sclerosis of the liver. The liver

PLATE 1

EXPERIMENTAL CALF E-64 (LEFT IN PICTURE) AND CONTROL CALF E-65 (TWINS)

Age, 177 days.

Weight of E-64, 181 pounds (52 per cent normal).

Weight of E-65, 318 pounds (93.1 per cent normal).

Height of E-64, 90.5 cm. (90.2 per cent normal).

Height of E-65, 97.6 cm. (97 per cent normal).

This picture shows the marked contrast in the size and condition of the twin calves E-64 and E-65 due to the fact that E-64 was on a vitamin A deficient diet, whereas E-65 received the same ration plus cod liver oil as a source of vitamin A. E-64 was totally blind when the above picture was taken, and xerosis of the eyes was starting.

of E-64 was tested for vitamin A storage by feeding to rats with the results shown in figure 10. One gram daily supplements of the fresh liver did not renew growth or heal xerophthalmia in young rats as did the liver of E-38 as shown in figure 6.

Control calf E-65. Freemartin with experimental calf E-64; E-65 was given a ration identical to that of E-64 and E-66 except that she received in addition 20 cc. of cod liver oil daily as a source of vitamin A. E-65 grew at a normal rate and showed no abnormal symptoms through-

out the experimental period. At three months of age there was a marked contrast in the appearance and vitality of E-65 and experimental calves E-63, E-64 and E-66. As shown in figure 9 E-65 had passed the experimental animals in weight at the age of 133 days even though she was considerably the smallest at birth. Plate 1 previously referred to, shows the contrast in E-64 and E-65 at 177 days. E-65 was taken off the experiment on the 183rd day when E-64 was killed.

Experiment calf E-66. Female; her dam received a ration similar to that of the dam of E-63. The growth by weight curve of E-66 is shown in figure 9. She was scouring on the 46th day. She stopped growing in weight at 80 days and 106 days her eyes and nose were starting to run. Her eyes became matterated and she was totally blind on the 139th day. She was very weak and scouring profusely. She died on the 140th day. Post-mortem examination showed pneumonia, nephritis, intestinal atrophy and changes in the rumen. As shown in figure 10, rat feeding tests failed to demonstrate any vitamin A in the liver of E-66.

DISCUSSION OF RESULTS

Vitamin A is shown to be an indispensable factor in the diet of calves. The characteristic symptoms of vitamin A deficiency occur in calves as they do in other species of animals. The practical significance of the results are hard to estimate. The three calves which showed unmistakable symptoms of vitamin A deficiency received rations quite low in vitamin A. Two calves receiving the same ration with cod liver oil added showed no abnormal symptoms whatever. No attempt was made to determine the amount of cod liver oil that would just protect the calves from showing symptoms of a deficiency. However, with the two calves in question, a daily consumption of about 20 cc. of cod liver oil which represented about 0.8 per cent by weight of the ration was sufficient for protection.

One calf consuming wheat straw as 40 per cent of the ration as the only source of vitamin A was able to make normal growth. Another calf on the same ration, but consuming less wheat straw developed symptoms of vitamin A deficiency. However, since the dam was stall-fed, the latter calf undoubtedly had a smaller vitamin A storage at birth than the other calf, whose dam received

pasture for the greater part of the gestation period. The vitamin A storage at birth is probably an important factor in the successful raising of calves.

It is a question whether ordinarily there is much danger of a deficiency approaching disaster in the rations of growing cattle. Calves are usually given whole milk and skimmilk until several months of age, and if they receive a roughage containing vitamin A in addition, there would seem to be an adequate supply of the factor. On the other hand, in areas where whole milk is sold and calves are raised on milk substitutes the chance of a vitamin A deficiency might be considerable. The danger would be augmented if the dams were stall-fed and consuming only dry feedstuffs. This somewhat artificial method of feeding and handling cattle is becoming more common.

While calves probably would seldom show symptoms of vitamin A deficiency such as xerophthalmia and running of the nose, there is every reason to believe that some of the earlier symptoms such as poor growth and a chronic condition of scours might exhibit themselves in the case of some calf rations. In this connection we might refer again to the work of Cramer (11) who believes that vitamins exert a positive action similar to that of hormones, for, when added to the diet of rats that had never suffered from any dietetic deficiency, they produced a stimulating effect on the processes of food adsorption from the intestines and growth. Also Sherman and Campbell (31) have concluded from their work with various proportions of milk in the diet that "evidently there is not only a line to be drawn, but a wide zone to be explored between adequate and optimum nutrition."

In the investigation reported in this paper it was found that calves suffering from vitamin A deficiency improved almost immediately when cod liver oil was given as a source of the vitamin. Especially was this improvement noted in the appetite, in the condition of the fecal matter, in vitality, and in growth. It is possible that many calves showing some of these symptoms might be benefited by the addition of a source of vitamin A, such as cod liver oil, to the ration. A supply of vitamin A approaching the "optimum" of Sherman and Campbell (31) would undoubtedly

aid calves in securing their maximum growth on the food consumed, and in preventing infections.

CONCLUSIONS

1. The experimental results with 9 calves show that vitamin A is an indispensable factor in the diet of calves.

2. The characteristic symptoms of vitamin A deficiency in other species of animals, including failure to grow, xerophthalmia, respiratory troubles, diarrhea and death, occur in herbivora, as represented by calves.

3. Cod liver oil feeding causes a resumption of growth and a disappearance of abnormal symptoms in calves declining on a vitamin A deficient ration.

4. Less than 1 per cent of cod liver oil in a ration otherwise practically free of vitamin A allows calves to grow normally.

5. Vitamin A is present in large amounts in the liver of calves fed normal rations but is absent from the liver of calves fed vitamin A deficient diets.

6. Wheat straw is a good source of vitamin A for ruminants.

7. White corn (Rustler White variety) and dried beet pulp are practically free of vitamin A.

8. Dried beet pulp can be used as a roughage for growing calves provided they receive adequate vitamin A in the other constituents of the ration.

9. Skimmilk powder oxidized by exposure to light and air until it becomes tallowy, is practically free of vitamin A.

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SWEETENED CONDENSED MILK

IV. A REFRACTOMETRIC METHOD FOR DETERMINING TOTAL SOLIDS*

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For many years the various types of refractometers have been extensively used in chemical analysis. These instruments have been so simplified and standardized that they have proved extremely convenient for many purposes.

The determination of the quantity of substance in solution is one of the most important forms of analysis to which refractive principles have been applied, refractive index increasing regularly with the percentage composition of the solution: Estimation of the quantity of alcohol in solution is perhaps the most familiar example. Reagents may be standardized with a fair degree of accuracy by the use of refractometric tables. Since the dry matter in milk serum is quite constant for normal milk, refractive index of the serum furnishes data from which conclusions can be drawn as to whether or not the milk has been watered. The refractometer has found wide employment in estimating the amount of sucrose in solution in sugar house work. The use of this instrument for the determination of dry matter in jams, jellies and marmalades (1) and in honey (2) has recently been recommended.

These are for the most part cases of true solutions. In a product such as condensed milk we have to do partly with true solution, but also with substances in colloidal solution, and, in mere suspension (fat). If, therefore, refraction is to be a meas-

ure of dry matter in such a case, it is necessary that the refractive index of liquids be affected in a regular manner by substances in colloidal solution and in suspension, as well as by those in true solution. Sufficient evidence is at hand for the validity of the hypothesis as applied to colloids: Wintgen (3) demonstrated a regular increase in refractive index with increase in concentration of colloidal As_2S_3, Sb_2S_3, silicic acid, molybdic acid, $Fe(OH)_3$ and tannin; while Reiss (4), Robertson (5) and others have given ample evidence of the possibility of determining the quantity of protein present in colloidal solution by means of refractive index.

Probably no one has ever claimed that a suspended substance, such as fat, might behave similarly, yet there is reason to predict that such would be the case; for it is well known that refractive index depends only on the size and number of molecules in the path of light, and is independent of the state of aggregation. Of course, there must be some limit; the suspended substance must be reasonably well divided. As will later be shown, the fat of condensed milk behaves in the manner predicted.

<center>EXPERIMENTAL</center>

A refractometer of the Abbe type was used. The adjustment was tested from time to time with pure water which should read 1.3330 at 20°C. Readings were always made at 20°, the temperature of the prisms being controlled by circulating water, and it was found best to have the sample at about that temperature before introducing the drops into the instrument.

Samples of condensed milk were obtained from the market, and also through the kindness of the Nestles Food Company, fresh from a vacuum pan in operation in the factory. All samples more than a few hours old were heated to get the lactose into solution; the following experiment shows the necessity for this:

A sample taken from the vacuum pan just at the finish of a batch was observed at intervals for several hours, making refractive index readings and noting the appearance under the

microscope. For the first few hours the refractive index remained constant, even after small crystals of lactose had appeared. However, after standing over night the refractive index had dropped from 1.4742 to 1.4732 at which time the lactose had crystallized to a considerable degree. But upon heating until all crystals had dissolved the refractive index went back to 1.4742.

Total solids were determined in most cases by the A. O. A. C. method, using ignited sea sand for drying residues; in some cases the determination was made on the Mojonnier machine.

TABLE 1

SAMPLE NUMBER	PER CENT TOTAL SOLIDS		REFRACTIVE INDEX AT 20°C.
	(1)	(2)	
1	70.55	70.53	1.4678
2	72.27	72.28	1.4710
3	72.39	72.42	1.4700
4	73.22	73.18	1.4729
5	73.38	73.34	1.4730
6	74.11	74.13	1.4751
7	74.13	74.13	1.4751
8	74.16	74.10	1.4757
9	74.37	74.37	1.4759
10	74.38	74.37	1.4759

CONDENSED MILK WITH 8 PER CENT FAT

As will be shown later, the percentage of fat considerably influences the relation between solids and refractive index, while the other main variable constituent—sugar—has practically no such effect.

Ten samples of 8 per cent milk of various brands were obtained from the market. Probably none was less than a month old. Duplicate determinations of total solids by the A. O. A. C. method, and the refractive indices are recorded in table 1.

Extraordinary care was taken in making determinations both for total solids and refractive index; in many cases two or more operators checked the results.

While other data were accumulated wholly in line with these

results, these alone were used in the calculations, and others will not be given.

By applying the method of the least squares to these data the following formula was derived:

$$T = 70 + 444 \ (n - 1.4658)$$

By means of this formula total solids (T) of any sample can be calculated when the refractive index at 20°C. (n) is known.

TABLE 2

For estimating from the refractive index the total solids in condensed whole milk with 8 per cent fat

REFRACTIVE INDEX AT 20°C.	PER CENT TOTAL SOLIDS
1.4600	67.42
1.4610	67.87
1.4620	68.31
1.4630	68.75
1.4640	69.19
1.4650	69.64
1.4660	70.08
1.4670	70.52
1.4680	70.96
1.4690	71.41
1.4700	71.86
1.4710	72.31
1.4720	72.75
1.4730	73.19
1.4740	73.63
1.4750	74.08
1.4760	74.52
1.4770	74.96
1.4780	75.41
1.4790	75.86

Also by the use of this formula a table was prepared by means of which the per cent of total solids may be read off when the refractive index is known (table 2). This should prove more convenient than the formula for regular use.

On several occasions access was had to a condensed milk factory while the condensing pans were in operation. Samples

were taken immediately after striking the batch and in some cases samples were drawn from the pan at intervals before the batch was finished. Total solids were run on a Mojonnier machine in the factory without checking the results. In table 3 will be found the refractive indices at 20°, total solids obtained therefrom applying the table, and also the total solids determined gravimetrically. Considering the fact that the determination of solids in sweetened condensed milk by any gravimetric method is a most difficult operation with great possibility of error, the comparisons obtained here are quite remarkable.

TABLE 3

BATCH	REFRACTIVE INDEX AT 20°C.	PER CENT TOTAL SOLIDS: REFRACTOM- ETER AND TABLE	PER CENT TOTAL ACIDS: MOJONNIER
A (finish)..............................	1.4749	74.04	74.08
B (1).................................	1.4674	70.60	70.93
B (finish)..............................	1.4706	72.13	72.18
C (1).................................	1.4713	72.44	72.80
C (finish)..............................	1.4731	73.23	73.58
D (1).................................	1.4611	67.91	67.95
D (2).................................	1.4649	69.60	69.43
D (3).................................	1.4676	70.78	70.76
D (4).................................	1.4688	71.31	71.52
D (5).................................	1.4722	72.84	72.41
D (finish)..............................	1.4735	73.41	73.63

SWEETENED CONDENSED SKIMMILK

As would be expected when a certain percentage of any one constituent is lowered and that of others correspondingly increased, and where the refractive powers of the interchanged constituents are different, then a different solids-refractive index relation would be expected.

A number of samples of skimmilk were procured. The fat content was not determined but it is entirely likely that some was present, and the amount variable. This may account for the slight discrepancy in some of the data. The total solids was determined by the A. O. A. C. method, also the refractive index at 20°C. The results are given in table 4.

TABLE 4

SAMPLE NUMBER	PER CENT TOTAL SOLIDS		REFRACTIVE INDEX AT 20°C.
	(1)	(2)	
1	70.97	71.05	1.4723
2	71.26	71.32	1.4720
3	71.30	71.35	1.4735
4	71.96	71.91	1.4747
5	72.16	72.08	1.4754
6	72.21	72.21	1.4757
7	72.30	72.39	1.4762
8	72.35	72.33	1.4750
9	72.44	72.44	1.4777
10	72.64	72.82	1.4767
11	73.78	73.78	1.4792
12	73.82	73.80	1.4787

TABLE 5

For estimating from the refractive index the total solids in skimmilk

REFRACTIVE INDEX AT 20°C.	PER CENT TOTAL SOLIDS
1.4680	69.29
1.4690	69.69
1.4700	70.08
1.4710	70.47
1.4720	70.86
1.4730	71.26
1.4740	71.65
1.4750	72.04
1.4760	72.43
1.4770	72.83
1.4780	73.22
1.4790	73.61
1.4800	74.00
1.4810	74.40
1.4820	74.79
1.4830	75.19

Upon resolving these results into a formula by the method of the least squares, the following is obtained:

$$T = 70 + 393\,(n - 1.4698)$$

As in the case of whole milk this provides a means for deter-

mining total solids from the refractive index of condensed skim-milk and also for the preparation of table 5.

Since the curves for both whole and skimmilk are almost exactly straight lines the tables can be extended by interpolation without error.

FUNDAMENTAL BASIS FOR THE RELATION BETWEEN TOTAL SOLIDS AND REFRACTIVE INDEX

It seemed desirable to learn whether or not the relationships herein established are based on sound theoretical principles. If it could be so proved then there would be entire justification for claiming that these formulas and tables will hold under all conditions.

Refraction is an additive property. Much data have been presented in the literature to show that the refractive power of a solution is equal to the sum of the refractive powers of its constituents. Of course, any changes in volume occurring when one substance is dissolved in another must be taken into account. This is done by introducing a factor for density. The formula generally used for comparing refractive powers is

$$\frac{n^2 - 1}{(n^2 + 2)d}$$

where n is the refractive index of the substance and d the density. This gives a refractive constant which is independent of the physical state of the substance, and of the temperature. If solute A is dissolved in solvent B then

$$\frac{n^2 - 1}{(n^2 + 2)d} \text{ (of } A) \times \%A + \frac{n^2 - 1}{(n^2 + 2)d} \text{ (of } B) \times \%B = \frac{n^2 - 1}{(n^2 + 2)d}$$

of the solution. This has been found to hold in any number of instances.

By making a complete analysis of condensed milk and obtaining the refractive constant of each constituent, it should be possible to determine whether or not the principle holds in this case.

The refractive constants of some of the milk constituents

can be found in the literature, others cannot: Water is given in many places as 0.20606; this is the figure used in this work. Sucrose is given by Browne (6) as 0.20614. Wiegner (7) obtained 0.1377 for the ash of milk. No value could be found for fat, but by taking the average of a large number of determinations made by others of refractive index and of density of milk fat a refractive constant of 0.2868 was obtained. Robertson (8) gives 1.675 as the refractive index of casein in solution, and 1.39 as the density. These figures give a refractive constant of 0.2703. The refractive index of solutions of albumin of various strengths was obtained from the work of Reiss and Robertson, and the densities from Chick and Martin (9) and from the Chemists' Year Book (Atack); with these figures an average refractive constant of 0.2494 was calculated.

No satisfactory figures could be found for lactose so that the determination was made experimentally: Several solutions of pure lactose were prepared, the refractive index determined to the fifth decimal place by means of a dipping refractometer and density with an accurate picnometer. The refractive constant of anhydrous lactose was found to be 0.20814.

A sample of condensed whole milk and one of skim were analyzed for sucrose, lactose, casein, albumin, fat, ash and moisture. The usual methods of analysis were followed except that albumin and casein had to be calculatd from the total protein since there is no way of separating heat coagulated albumin from casein; after determining total protein, 20 per cent was taken as albumin and 80 per cent as casein. Although the value for moisture was taken as the difference between the sum of the other constituents and 100 per cent, the figures were quite close to those obtained by direct determination.

Table 6 gives for one sample of whole milk the percentage of each constituent, the refractive constant of each, and the value obtained by multiplying these two together. The sum of the last named values is seen to be 0.2165 (a calculated refractive constant for the sample). The refractive constant was determined also from the refractive index of the sample of condensed milk (1.4750), and the density (1.3050), which were found by

experiment.   These values give a refractive constant of 0.2158
These results compare favorably, being well within the limits
of error, since the refractive constants employed for some of the
constituents may be in slight error, and the method of deter-
mining albumin and casein is by no means unobjectionable.
Also no account is taken of some of the constituents that must
be present, such as citric acid.

TABLE 6

CONSTITUENT	PER CENT (P)	REFRACTIVE CONSTANT (K)	P × K
Water	26.56	0.20606	0.054729
Sucrose	45.1	0.20614	0.092969
Lactose (anhydrous)	11.4	0.20814	0.023727
Casein	6.04	0.2703	0.016326
Albumin	1.50	0.2494	0.003741
Fat	8.09	0.2868	0.023202
Ash	1.31	0.1377	0.001803
Sum			0.216497

TABLE 7

CONSTITUENT	PER CENT (P)	REFRACTIVE CONSTANT (K)	P × K
Water	27.82	0.20606	0.057326
Sucrose	45.70	0.20614	0.094206
Lactose	14.30	0.20814	0.029764
Casein	6.67	0.2703	0.018029
Albumin	2.25	0.2494	0.005612
Fat	1.26	0.2868	0.003613
Ash	2.08	0.1377	0.002864
Sum			0.211414

In exactly the same way a sample of condensed skimmilk
was carried through.   Table 7 gives the results.

Refractive index of this sample was found to be 1.4777 and
the density 1.347.   This gives 0.2100 for the experimental
refractive constant, which again compares favorably with the
figure 0.2114 obtained by calculation.   All the sources of error
mentioned when considering whole milk are likely to be found
here also.

This section of the work shows that the refractive power of condensed milk follows the general law of true solutions. It must, therefore, be concluded that the estimation of total solids from refractive index readings is founded on scientific principles.

The fact that the fat contributes its share to the refractive power of the condensed milk in exactly the same way as do the constituents in true and colloidal solution, shows, indeed, that the state of aggregation of the molecules does not influence the additive principle.

It should be pointed out at this point, however, that difficulty may be experienced in applying this theory in many cases. Unless the refractive index of the suspended substance is very near that of the suspending medium there may be such a dispersion of light that the shadow edge in the refractometer is diffused so as to make it impossible to get a satisfactory reading. Fortunately the conditions necessary for obtaining a good reading are fulfilled in sweetened condensed milk. But with evaporated milk, whole milk and ice cream mixes, it is otherwise; it is not possible with any degree of accuracy to read any of these products for the reasons stated. A method such as is here worked out for condensed milk cannot be devised for these other milk products.

As has been pointed out, when lactose crystals are very small, the true refractive index may still be obtained; it is only when the crystals have grown to their usual maximum size that a low reading results. Apparently, therefore, there is a limit beyond which there may be an error due to the large size of the fat globules. Of the many samples of condensed milk examined in this work there was no suspicion of error in any one from this cause; but it is possible that through fat separation, for instance, the globules may become so agglomerated as to cause an incorrect reading.

As has already been suggested, it is important that the percentage of fat in the sample be known within fairly close limits, while a variation in the sugar content is practically without influence. By referring to table 6 it will be seen why this is the case. The refractive constant of sucrose is very close to

that of the condensed milk itself while that of fat is far away. A variation in the standardization for sugar within several per cent would not alter the relation between solids and refractive index as much as a considerably smaller variation in fat. It is recommended, therefore, that if there be 9 per cent, or more, fat, such as is often found in condensed milk manufactured in Europe, a new formula and tables be worked out.

PROCEDURE FOR THE ESTIMATION OF TOTAL SOLIDS IN SWEETENED CONDENSED MILK WITH AN ABBE REFRACTOMETER

If the specimen has been undisturbed for some time, some of the crystallized lactose will have collected at the bottom. In this case, warm by placing the container in hot water, then transfer the entire contents to a mortar and mix well.

Place a few cubic centimeters in a thick walled test tube. Heat by immersing in boiling water until no more lactose crystals are observed under the microscope. During this operation it is important that the test tube be kept stoppered to prevent evaporation, but the stopper may be removed for an instant from time to time to release the pressure. (The needle-like crystals of calcium citrate may remain undissolved without affecting the results.) If the amount taken is not too large, and if the liquid is mixed frequently by inverting the tube, this operation should require five minutes. Cool to about 20°C. by immersion in cold water, then place tubes in a tempering bath held near that temperature for ten minutes.

The temperature of the refractometer should be controlled at 20°C. by circulating water. Examine two or three different drops in the instrument. The different readings should check exactly.

If the sample is an 8 per cent fat product refer to table 2, if skim, use table 5, reading off the total solids from the refractive index. Interpolate where necessary.

Where a specimen is drawn fresh from the vacuum pan, the preliminary treatment is unnecessary. Merely cool the sample to 20° and read.

SUMMARY AND CONCLUSIONS

The successful use of the refractometer in estimating the dry matter in various liquids led to the conclusion that a process might be worked out for sweetened condensed milk.

The refractive indices of a number of samples of condensed milk with 8 per cent fat were determined, and also careful analyses made for total solids, gravimetrically. From these results a formula was derived by means of which the total solids in any sample can be determined from the refractive index. By employing this formula a table was prepared to be used for a similar purpose.

Refractometric readings were made on a large number of samples taken directly from the vacuum pan, and from these figures the total solids was read off on the table. Total solids was also determined on the Mojonnier machine. The comparisons were found to be excellent considering the liability of error which is large in the gravimetric determination of total solids.

From results obtained on a number of samples of condensed skimmilk a formula and table were worked out in the same way and for the same purpose as with whole milk.

The refractive constant of condensed milk is shown to be equal to the sum of the products obtained by multiplying the refractive constant of each constituent by its percentage. This is the fulfillment of a law which holds generally for true and colloidal solutions, and is proof that the solids-refractive index relation applied to condensed milk is scientific and dependable.

The fact that the fat of condensed milk behaves in this respect as do those substances in true and colloidal solution leads to the conclusion that an agglomeration of molecules in masses even beyond the colloidal size influences the refractive index of a liquid exactly as if the molecules were dispersed in true solution. Necessary conditions, however, are that the suspended particles be not too enormous, and that the refractive index of the suspending medium be fairly near that of the suspended substance.

A formula or table giving the relation between refractive index and total solids will hold even if the sucrose content should vary within wide limits, but the fat must not be far from the percentage for which the table and formula are prepared.

Not only should the refractometric method prove useful in estimating total solids in sweetened condensed milk as a matter of laboratory routine, but it may be, and has been, used as a means of determining the finishing point of batches in the factory. Near the finish of the batch samples are drawn from the pan, cooled, and read off on the refractometer with such ease and speed that the proper point of striking is accurately determined.

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GENETICS OF BREEDING BETTER DAIRY STOCK*

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Better dairy stock suggests animals meeting the requirements of the breed association to which they may belong, free from physical defects, uniformly high in the production of milk and butter-fat, a conformation pleasing to the eye, and consistent in breeding performance. Genetic research has as one of its objectives the analysis of the laws of inheritance governing these characters. There would seem to be little doubt that such a genetic analysis will furnish the means by which the practical objective, better dairy stock, will be attained, as already we are indebted to the advances in this field for taking the gamble out of and making the results more consistent in breeding better varieties of many things.[1]

The most obvious application of elementary genetics to these problems is that offered by the requirement of the black breeds that animals to be registered in them shall be black in color and nothing else. Spillman (1), Van Damme (2), Wilson (3), Lloyd-Jones and Evvard (4), Gowen (5), Cole and Jones (6) and Templeton (7) have presented evidence to show the dominance of black over red with the segregation of black from red in a three to one ratio in the second generation. Many of the black breeds have originated from crosses of black on red animals, with subsequent selection of the black animals. Under such conditions the red color may lie hidden in the germ cells of the black coated animals for many generations waiting for a mating which shall bring two reds together in the fertilized egg. This egg is then capable of giving rise to a red animal from purebred black parents, an

animal which, however much it may be worth, cannot be registered.   Cases of this sort are not only disappointments but serious economic losses.   Our knowledge of genetics shows that both black parents are equally responsible for the production of the red animal.   The parents' germ cells, sperms or eggs, are in fact half of them carrying the red producing substance, thus
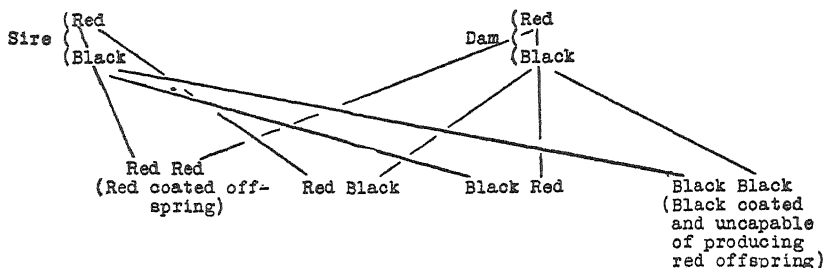
The damage resulting to a herd from such a mating is difficult to remedy as the production and ultimate loss of the red calf is but the least of it.   On the average one out of two of the offspring of such a black sire carrying red also carries the red factor. Such animals on being properly mated are also capable of having red offspring.   An animal of this sort is consequently a menace to a herd.   In view of this fact it is an open question if the bulls purchased or sold for breeding within the black breeds should not be tested to determine first whether or not these bulls are truly black in their breeding.   Our present-day knowledge of inheritance shows that such a test may best be made by breeding the black bull to red cows—if any of the offspring are red the bull should be discarded as he would be known to carry the red factor.

Several of the physical defects found in cattle are now known to be or there is good presumptive evidence that they are due to inherited factors.   Such physical defects are congenital cataract (8), defective hair and teeth (9), notched ears (10), abnormal number of toes (11).   The inheritance of these defects appears to be of the simple Mendelian type, the elimination of them from the breeding strain being easily possible with the knowledge of how they are inherited.

INHERITANCE OF MILK PRODUCTION

The now clearly recognized differences which exist between the production of some of the breeds, all point to the conclusion that milk production is innate and hereditarily determined between certain breeds.   Such reasoning has led certain dairymen to make crosses between breeds in the attempt to combine the high milk production of one breed with high butter-fat percentage of another.   It is now known that such crosses are based on false reasoning for while the first generation offspring will generally show higher production than the lower producing breed, it will not show any higher production than the high producing breeds. Furthermore, while the butter-fat percentage of the first generation offspring will be higher than that of the low test breeds it is, in general, considerably lower than that of the high test breeds. These results lead to a total butter-fat production which is about equal or slightly more than the butter-fat yield of the two crossed breeds.   Furthermore than this, we know that such crosses lead to second generation offspring which are extremely variable in production and in type.   In other words, the dairyman has before him the prospect of years of selection before a stable type is reached with but doubtful results at the end as far as his object of combining high milk production with high butter-fat percentage is concerned.   What the dairyman is interested in, therefore, is something more than crosses between breeds.   He wants to know whether the differences observed in milk production between different individuals of the same breed are inherited. Should this be true, he may start with a fairly stable type and have to select and breed for but relatively few characters, thus making for a greatly increased chance of success.

The general observation that milk yield differs between strains has indicated that at its foundation many of these differences in producing ability are also hereditary.   But how and what are the limitations?   For plainly this is not a simple matter, for if it were none of our cows would produce less than 10,000 or 12,000 pounds of milk.   The first step to be taken toward the improvement of dairy stock by breeding is then to find out the way in which the

variations or differences in producing ability behave in their
inheritance.

The problem[2] of breeding better dairy stock is not so much to
produce animals of higher production than those which we have
today as it is to be able to reproduce the best of our present-
day stock uniformly and at will.   In other words, we wish to
know what are the significant facts to be taken into consideration
in breeding such stock and what items are of but trivial impor-
tance.   This problem is made doubly difficult by the fact that it is
impossible to measure the character, milk production or butter-
fat percentage, in the sire.   It is, in fact, only possible to arrive
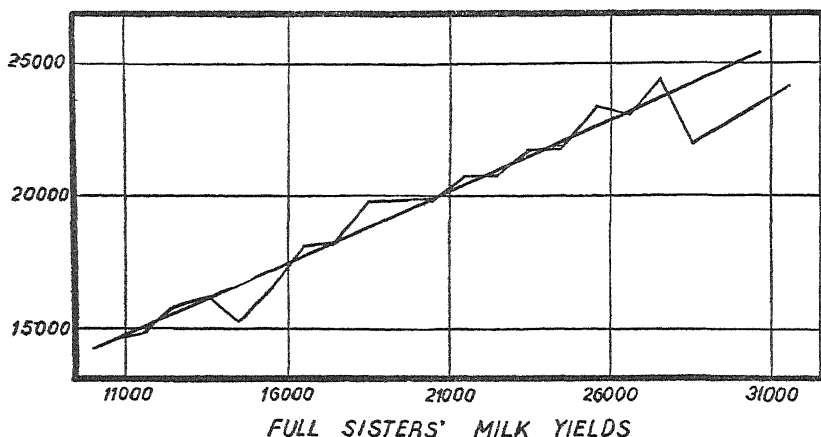
FIG. 1. RELATION BETWEEN THE MILK PRODUCTION OF FULL SISTERS ON A MATURE
FORM BASIS FOR THE HOLSTEIN-FRIESIAN ADVANCED REGISTRY COWS

at a measure of the influence of the sire on the production of his
offspring by indirect means.

Full sisters have a common sire and common dam. Half sisters
have either the sire or the dam in common. If the sire alone
determines the production of his daughters the above facts would
lead to the expectation that full sisters would resemble each other
in their production; that half sisters with a common sire would
resemble each other in production and to the same degree that
full sisters are correlated; that half sisters with a common dam

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[2] For a more complete discussion of this important problem see Milk Secretion,
The Williams & Wilkins Company, Baltimore.

would not resemble each other.   If on the other hand, both the sire and dam determine the production of their daughters, the full sisters' production would be fairly closely correlated; the half sisters' productions correlated but to a less degree than the full sisters.   The correlation between half sisters should be of the same order of strength whether these half sisters had a common sire or dam.   If, as the last alternative, the dam determined the production of her daughters with the sire having no influence on production, the expected relations would be, full sisters' production correlated; half sisters' productions where the sire was the common parent not correlated; half sisters with a common dam correlated in their production to the same degree as the full sisters.   The actual results derived from a comparison of the production of full and half sisters for Holstein-Friesian cattle are shown in table 1.

TABLE 1

Correlation coefficients for the relation of the milk yields and butter-fat percentages of sisters, Holstein-Friesian Advanced Registry

	MILK YIELD	BUTTER-FAT PERCENTAGE
Full sisters..	0.55±0.03	0.46±0.03
Half sisters (common sire).....................	0.36±0.02	0.37±0.02
Half sisters (common dam).....................	0.38±0.03	0.22±0.04

The full sisters resemble each other more closely in their milk yields or butter-fat percentages than the half sisters.   The half sisters with a common sire or dam appear to resemble each other to about the same degree.   The experimental facts thus accord with the hypothesis that the sire and dam are both responsible for the milk yields or butter-fat percentages of their daughters. This conclusion is also supported by similar evidence from Guernsey cattle.   The stringency of selection for animals entering the Guernsey Advanced Registry appears to have been greater than it was for those animals entering the Holstein-Friesian Advanced Registry.   This selection is known to affect the correlation coefficient.   The correlation coefficients for the Guernsey are consequently not directly comparable with those of the Holstein-Friesian breed but should in all probability be somewhat lower.

This differential effect should not materially influence the relative results between full sisters and half sisters. The correlation coefficients describing these relations are given in table 2.

Although the correlation coefficients are lower for the Guernsey Advanced Registry cattle the full sisters stand in the same relation to the half sisters as that displayed in table 1. The full sisters resemble each other more closely in their milk yields and butter-fat percentages than the half sisters. The half sisters, on the other hand, have practically the same degree of resemblance. Thus, here again, there is agreement between experimental facts and the hypothesis that the sire and dam are both responsible for the milk yields and butter-fat percentages of their daughters.

This conclusion is also borne out by such critical results

TABLE 2

Correlation coefficients for the relation of the milk yields and butter-fat percentages of sisters, Guernsey Advanced Registry cattle

	MILK YIELD	BUTTER-FAT PERCENTAGE
Full sisters......................................	0.41±.02	0.44±.02
Half sisters (common sire).....................	0.13±.02	0.17±.01
Half sisters (common dam)....................	0.15±.02	0.19±.01

as those obtained from the wide crosses of animals of the high milking breeds with those of the low milking breeds, or of animals of the high butter-fat test with those of the low butter-fat tests. The results appear to be comparable, either way the cross is made, thus indicating an equal effect of the sire and dam on the production of the offspring.

The relation which exists between the milk production or butter-fat percentage of full sisters as contrasted with that of half sisters shows that from a practical breeding standpoint, and as a means of guiding breeding operations, the milk yield or butter-fat percentage of one daughter should be considered very carefully in determining whether or not it is desirable to repeat this mating as such a repetition will tend to beget daughters of similar production. If the daughter is an undesirable cow from a production standpoint, it would appear as if it would be better

to change either the sire or dam, as by so doing the daughter's production would probably be increased.

The influence of the dam on the milk production of her daughters may be measured directly by comparing the productions of the daughters with those of the dams. On making this comparison for Holstein-Friesian cattle, a correlation is found between the production of daughter and dam in milk yield or in
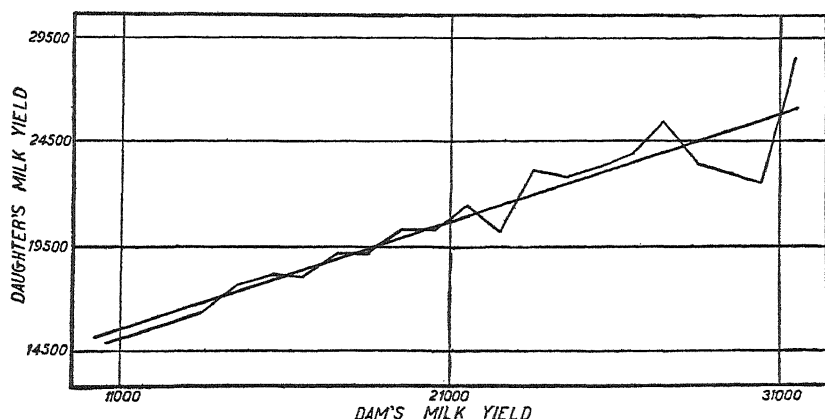
FIG. 2. RELATION BETWEEN THE MATURE FORM PRODUCTION OF DAUGHTERS AND THEIR DAMS FOR HOLSTEIN-FRIESIAN ADVANCED REGISTRY CATTLE

TABLE 3

Correlation coefficients measuring the relation of daughters' and dams' milk yields and butter-fat percentages, Holstein-Friesian and Guernsey Advanced Registries

BREED	MILK YIELD	BUTTER-FAT PERCENTAGE
Holstein-Friesian..............................	0.50±0.02	0.41±0.02
Guernsey..	0.36±0.02	0.42±0.02

butter-fat percentage. A similar, although lower, correlation is also found to exist between the productions of daughters and dams as found in the Guernsey Advanced Registry. These correlations are shown in table 3.

Table 3 shows that there is a fairly close relation between the milk productions and butter-fat percentages of daughter and dam in either the Holstein-Friesian or Guernsey Advanced Registry cattle. This relationship is slightly less than that found between

full sisters and more than that found for half sisters. The
production record of a dam is consequently of considerable sig-
nificance in breeding and as a guide to breeding operations.

The amount of influence that the sire exerts on the production
of his daughters may be determined from the fact that for any
given sire, his daughters must lie within one array or column of
the correlation table. The relation of the variation in produc-
tion found for such an array of daughters to the variation in
production found for all daughters makes it possible to calculate
the correlation coefficient between the production of the daughters
and that of the sire, even though it is impossible to measure the
sire's production. When this calculation is completed the Hol-
stein-Friesian sire has a correlation coefficient between the produc-
tion of his daughters and what his might be of 0.52 for milk and
0.53 for butter-fat percentage. These correlation coefficients are
practically identical with those found in the same breed for the
relation of the productions of daughter and dam. In view of
these facts it is seen that on a quantitative basis the sire is equally
important with the dam in determining their offspring's milk
production or butter-fat percentage.

The influence of the ancestors further removed is of particular
importance in view of the frequent difficulty of obtaining records
on animals of the first generation. For the sire these records
must be those of the progeny, for the dam the records may be
either those of the progeny or her own records for production.
Progeny records are of course extremely difficult to obtain unless
the parents are quite old.

The interrelation between the production of the granddams,
maternal and paternal, with the production of their grand-
daughters is shown in table 4.

The data of table 4 show that there is a much closer relation
between the production of the daughter and dam as contrasted
with the relation which is found between the daughter and grand-
dam whether the granddam is on the maternal or paternal side.
This conclusion is supported by both the Guernsey and the Hol-
stein-Friesian data. It is true for both milk yield and butter-
fat percentage. The grandparents have about an equal relation

between their milk yields and those of their granddaughters or
between their butter-fat percentages and those of their grand-
daughters.   In view of these facts it may be concluded that as a
basis of constructive breeding, the dams' records are at least
twice as important as the granddams' records and that the
granddams' records are of about equal importance in indicating
the probable production of the daughter and granddaughter.
In the Guernsey data it is obvious that milk production or butter-
fat percentage are practically equally inherited from the grand-
parent.   In the Holstein-Friesian data the relation between the
milk yields of the grandparents with those of the granddaughters
is greater than that found for the butter-fat percentages of these

TABLE 4

*Relation of parents' and grandparents' performance records to those of their daughters
and granddaughters*

BREED AND ANCESTOR	MILK YIELD	BUTTER-FAT PERCENTAGE
Guernsey:		
Dam..	0.36±0.02	0.42±0.02
Paternal granddam...........................	0.16±0.01	0.15±0.01
Maternal granddam...........................	0.20±0.02	0.20±0.02
Holstein-Friesian:		
Dam..	0.50±0.02	0.41±0.02
Paternal granddam...........................	0.26±0.04	0.09±0.04
Maternal granddam...........................	0.31±0.05	0.19±0.05

same relatives.   The differences are however not significant in
view of their probable errors.   These facts may be brought out
very nicely by the use of graphical methods.   Figure 3 shows the
average milk yields of the daughters for fixed milk yields of the
dam and granddam.

   Figure 3 shows that as the milk yield of the dam increases the
milk yield of the daughters on the average also increases.   This
increase is more pronounced than the increase found in the
granddaughters' milk production with a corresponding increase
in the granddam's milk production.   On a numerical scale an
increase of a thousand pounds in the dam's milk production
results on the average in a corresponding increase of *367* pounds
in the daughters' milk production.   An increase of 1000 pounds
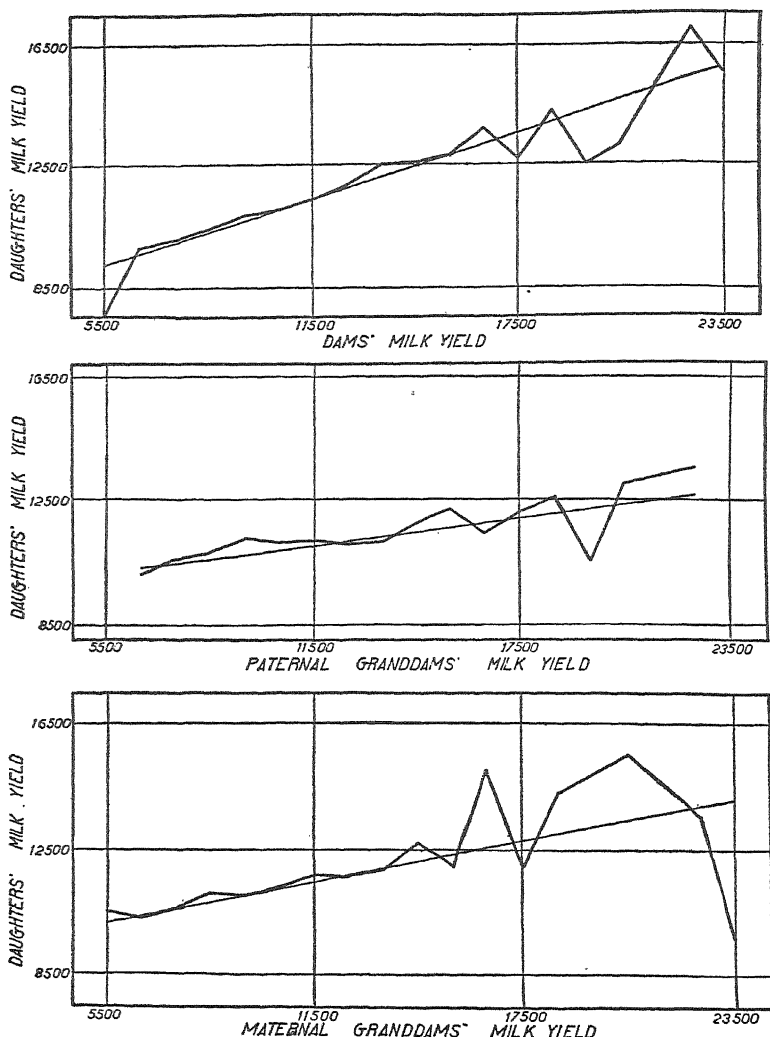
FIG. 3. AVERAGE MILK YIELDS OF DAUGHTERS OR GRANDDAUGHTERS FOR DAMS
OR GRANDDAMS IN THE GUERNSEY ADVANCED REGISTRY

The upper graph shows the relation between the daughter and dam. The middle graph the relation between granddaughter and paternal granddam. The lower graph the relation between granddaughter and maternal granddam. Examination of these graphs shows that for either type of ancestors an increase in the milk production results in an increased average production of their daughters or granddaughters. Contrasting the average production of the daughters for an increase in the production of the dams as compared with an average production of the daughters for a like increase in the production of the granddam, it is found that a given increase in the dam's production results in a much more marked average increase in the daughter's production, in fact nearly twice as great an increase in production as that found for granddam and granddaughter.

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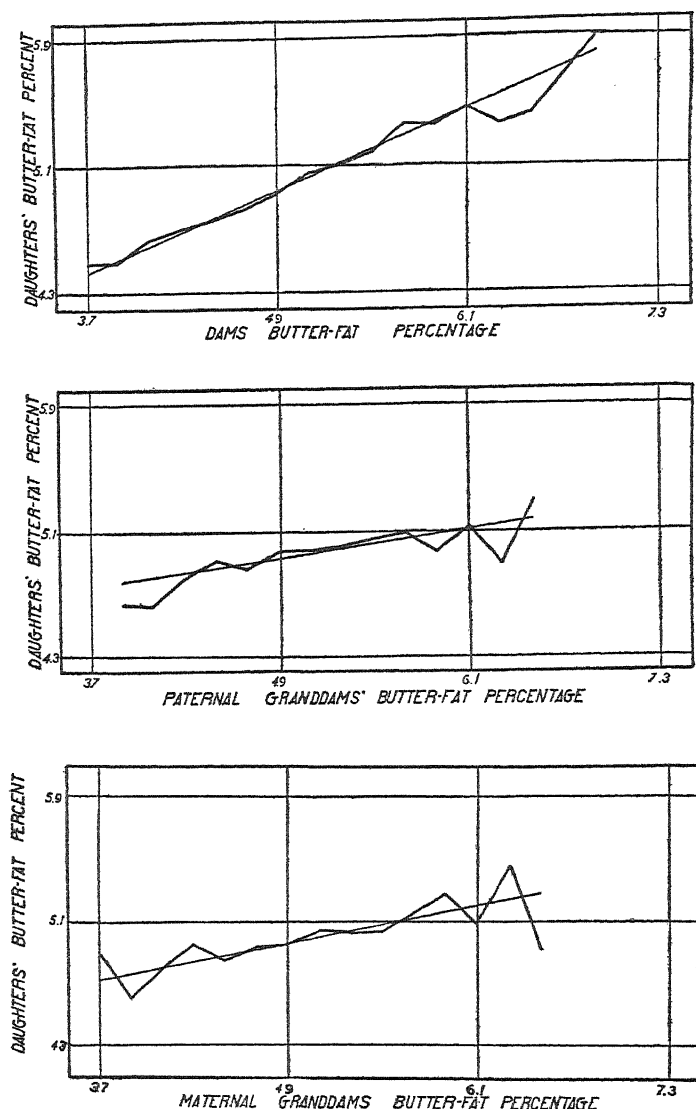
FIG. 4. RELATION BETWEEN THE BUTTER-FAT PERCENTAGES OF DAM OR GRANDDAM
AND THEIR DAUGHTERS OR GRANDDAUGHTERS

These figures are drawn by the same scale. The parents production is shown
in the abscissae of the figure, the ordinates represent the average production
of the daughters and granddaughters. The figure shows clearly that there is a
much greater relation between the dam's and daughter's butter-fat percentage
as contrasted with that of granddam and granddaughter.

of milk in the paternal granddam's production results in an
average increase for the daughters' of *143* pounds. An increase
of 1000 pounds for the maternal granddams' results in an average
increase of *224* pounds in the granddaughters' milk production.
Thus the dam's influence on production is clearly much more
marked than the influence of the grandparents. The grand-
parents differ in their influence perhaps significantly although this
appears doubtful.

Figure 4 shows the same relation for the butter-fat percentages.

Figure 4 shows that the dam's butter-fat percentage predicts
the daughters' butter-fat percentage much more accurately than
the granddam's butter-fat test predicts that of the grand-
daughters. On a numerical scale it is found that for an increase
of 1 per cent in the dam's butter-fat percentage there is a

TABLE 5

Correlation coefficients between the milk yields and butter-fat percentages of cousins,
Holstein-Friesian breed

COMMON GRANDPARENT	MILK YIELD	BUTTER-FAT PERCENTAGE
Paternal grandsire...........................	0.01 ± 0.03	0.12 ± 0.03
Paternal granddam...........................	0.17 ± 0.05	0.21 ± 0.04
Maternal grandsire...........................	0.21 ± 0.02	0.22 ± 0.02
Maternal granddam...........................	0.23 ± 0.04	0.24 ± 0.04

corresponding increase of *0.43* per cent in the daughters' butter-
fat percentage. For an increase of 1 per cent in the paternal
granddam's butter-fat percentage there is a corresponding average
increase in the daughters' butter-fat percentage of *0.16* per cent.
For an increase of 1 per cent in the maternal granddam's
butter-fat percentage the increase in the daughters' butter-fat
percentage is *0.20* per cent. The parents exert about twice the
influence on the offspring's production as is exerted by the
grandparents.

Among the ancestral combinations used in dairy cattle breed-
ing is that of cousins. The importance of this combination comes
from the habit of giving a good deal of weight to production
records of a brother's daughters in selecting a bull for a herd
sire. The daughters of such a bull would, of course, be cousins

to the daughters of his brother. The study of this problem shows that there is a small degree of relationship between the production, either in milk yield or butter-fat percentage of such cousins. The results for the Holstein-Friesian breed are shown in table 5.

Table 5 shows that there is a small degree of relationship between the production of cousins. This relationship is less for cousins with a common paternal grandsire than for those with other common grandparents. The difference is not significant. The degree of relationship between the milk yield of cousins appears to be of about the same order of magnitude as that for the butter-fat percentages of these same cousins. The average amount of this relationship is *0.16* for milk yield and *0.20* for butter-fat percentage. The production of a brother's daughters

TABLE 6

Correlation coefficients between the productivity of aunt and niece

VARIABLE	CORRELATION COEFFICIENT
Milk yield	0.26±0.02
Butter-fat percentage	0.18±0.02
Butter-fat	0.29±0.02

is consequently a slight indication of what the production of the herd sire may be.

Another important ancestral combination used extensively in guiding breeding operations is that of aunt and niece. This combination comes into use in the selection of a herd bull because of the belief that his daughters will duplicate what his sisters have done. The relationship between the sire's daughters and his son's daughters is that of aunt and niece. A comparison of the production records of aunt and niece leads to the correlation coefficients shown in table 6. The data is for Guernsey Advanced Registry cattle.

Table 6 shows that there is a significant relation between the production of aunt and niece in milk yield, butter-fat percentage, or butter-fat. The differences between these correlations are of doubtful significance indicating that the relationship is about

of equal strength between the productions of aunt and niece in
any of these items. Such results bear out the conclusion that
milk yield, butter-fat percentage, and butter-fat are equally
inherited and that the sire plays a fairly important part in this
inheritance. The degree of relationship is practically that found
for cousins. The record of an aunt or a cousin is consequently
of about equal importance in indicating the probable production
of any given cow.

The significance of these results for practical breeding may be
stated thus: It is important in constructive breeding to take
into consideration; first, the record of the bull's progeny; second,
the record of his dam; third, but weighing it to a still less degree,
the record of his sire's daughters or the bull's own half sisters.
When these records are obtained and carefully considered they
furnish about all the information which is valuable in selecting a
herd sire. In view of the difficulty in getting many of these
records it is an open question as to whether a herd sire should
ever be purchased. It is in fact likely that more progress in
building up the breed's production would be made by selecting
the best producing and transmitting cow in the dairyman's barn
to furnish the future herd sire. The significant records for a
cow are in order of importance: first, her full sister's record;
second, the record of her dam; third, the record of her half sister;
fourth, the record of her granddam; fifth, the record of her aunt;
and sixth, the record of her cousins. Other production records
have doubtful significance in indicating what the cow's produc-
tive capacity will be. From these facts it is obviously much
easier to select cows than it is to select bulls for prepotency in
transmitting milk production or butter-fat percentage to their
offspring.

Conformation has for many years been the major criterion
by which dairy breeding stock is selected. This practice is
based on the following ideas. First that conformation shows the
probable production of the cow, second that conformation of the
bull was transmitted to his offspring and therefore in a sense
showed what this offspring's probable production would be.
Within recent years data on this problem of some importance

have been collected and analyzed. The first problem does not in a sense belong in the genetic field. However, it is so intimately linked with breeding for production that it is important to review the evidence to form a foundation on which to study the breeding problem.

Conformation is found to be an important element influencing markedly the production of a cow. This influence is due to the fact that both conformation and milk production are closely associated with the age of the cow. If selection is practiced it is really a double selection based partly on conformation and partly on age. About half of the relation which exists between

TABLE 7

Conformation and productivity in Holstein-Friesian cattle under five and one-half years of age

CHARACTERS	MILK YIELD	
	Age variable	Age constant
Age	0.65	
Shoulder height	0.36	0.11
Hip height	0.36	0.14
Body length	0.58	0.26
Rump length	0.39	0.13
Body width	0.52	0.19
Thurl width	0.15	−0.06
Body girth	0.41	0.12
Weight	0.65	0.35

conformation and milk production is due to age and the other half is due to the differential growth which exists between cattle of the same age. Table 7 brings out this point for Holstein-Friesian cattle. The same general conclusion is also to be derived from a much more extensive and adequate series of data on Jersey animals.

The results of table 7 show that during the early years, age is of the most importance, much more important than conformation, in controlling milk yield. Body weight alone approaches the importance of age in controlling milk production. Besides this measurement other measurements indicating the relative size of the animal are also important. They are, however, closely

associated with weight so that in the long run weight appears to be the most important part of conformation to which consideration should be given in selecting dairy cows.

The score card data on Jersey cattle indicates the same general conclusion and supplements it in that it shows the udder condition in size and the characteristics of the milk veins to be very important elements of conformation in relation to milk production. The work of Aldrich and Dana bear out this conclusion in showing milk veins to be quite important elements of conformation as related to production. The data may, therefore, be summed up by saying that weight as a measure of size of the cow and the characteristics of the udder and milk veins are the important elements in indicating production.

The practice of the dairyman in selecting cattle for his herd on the basis of conformation in the belief that these animals would be capable of transmitting this favorable conformation to their offspring, has only recently been analyzed to establish whether this proposition were true or false. Thanks to the initiative of the American Jersey Cattle Club data are now available to show that the relative size of the cow is dependent to some degree on the relative size of the sire. If the sire is large the daughter is apt to be large. The degree of correlation which exists between the daughter's size and the sire's size is of about half the order of magnitude found between the milk production of a dam and that of her daughters. Thus to a limited degree the conformation of a sire is transmitted to his daughters.

The equally important hypothesis on which the dairyman manages his breeding herd, that the sire's conformation to some degree predicts the daughter's milk production, has now also been approached through the analysis of these same data collected by the American Jersey Cattle Club. The results of this analysis show that the item, weight of the sire, has a relation to the daughter's milk production. The larger the sire's weight the more the milk yield of the daughter. This relation is only about half of that found for the relation of the dam's and daughters' milk yield. It, furthermore, has no relation to the butter-fat percentage that the daughter is able to give. The other items of

conformation were not found to be particularly important in indicating the daughter's probable production. It is thus clear that conformation as a basis of selecting and breeding for milk production is of much less importance than the actual record of the ancestors within the first and second generations of the pedigree.

The significant points which genetics has thus far been able to analyze and present to the dairy cattle breeder interested in the intelligent betterment of his herd may be briefly reviewed as follows: The Mendelian analysis of coat color and of certain of the physical defects found in cattle has shown that by the use of of simple factor hypotheses it is possible to eliminate these defects and make for a more uniformly breeding animal whose characteristics will meet the breed association requirements. It has been possible to show that the production of the near relatives is much more important than that of those further removed. The significance of these results in selecting a bull for herd sire may be stated thus.

It is important to take into consideration: first, the record of the bull's progeny; second, the records of his dam; third, and weighting to a less degree the record of his sire's daughters or the bull's own half sisters. Unfortunately, it is very difficult to obtain these records on animals other than those raised by the breeder himself. In view of this difficulty it would frequently be to the breeder's interest to raise his own herd sire rather than to purchase an animal lacking such information. The more significant records for the cow are in order of importance: first, her full sisters' records; second, the record of her dam; third, the records of her half sisters; fourth, the records her granddams; fifth, the records of her aunts, and sixth, the records of her cousins. The other records have but doubtful significance in indicating what the cow's productive capacity will be. The variety of ancestors from which records may be obtained in selecting a cow make it clear that it is much easier to select females than it is to select males in replacing animals within the herd. Conformation is shown to have a relation to production, such that the size of the cow and the size of her udder and mammary development,

together with wedge shaped form are of importance in indicating the cow's probable productivity. It is further shown that the conformation of the sire is transmitted to his daughters to a limited degree. If the sire is large the daughter is apt to be large. The amount of relationship, however, on the whole is relatively small. The sire's conformation effects the daughters' conformation only in so far as the item of weight is concerned. It also appears that the most important item of the sire's conformation in affecting his daughters' milk production is that of weight. The larger the sire in weight, the more the probable milk production of the daughter will be. This relationship is, however, only about one-half of that found for the relation of the milk yields of dam and daughters so that it may be concluded that conformation is in no way a substitute for actual production records of the parents or grandparents of the animals purchased or bred in the maintenance of a productive herd.

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PHYSICO-CHEMICAL FACTORS INFLUENCING CREAM RISING

II. RELATION OF PLASMA COLLOIDS TO PASTEURIZATION EFFECTS*

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It was shown in the first paper of this series (1) that variations in cream rising of cow's milk seem to be due primarily to certain changes in the dispersion medium, i.e., the plasma of the milk. In raw milk especially it was shown that the standardization of a given lot of skim milk with cream having either large or small fat globules resulted in cream layers of nearly uniform depth. Pasteurization, also, seemed to affect the milk plasma primarily, resulting in less exhaustive creaming and a closer packing of the fat globules in the cream layer.

The detrimental effect of pasteurization on creaming has been variously explained. According to Hunziker (2) there are two factors involved, (a) a coagulation of lactalbumin to form a sort of network that hinders the rise of fat globules, (b) a reduction in the ability of the fat to carry non-fatty constituents into the cream. So far as the first factor is concerned, the experiments of Freudenreich (3), Rupp (4), Weinlig (5), Grimmer, Kurtenacker, and Berg (6), Steiner (7), and Hiebenthal (8) indicate clearly that appreciable coagulation of lactalbumin does not occur under the influence of such time and temperature factors as seriously affect creaming. Rahn (9) has, in fact, effectively disposed of this theory by restoring the normal creaming capacity of boiled milk through the addition of colloidal material, such as gelatin or gum.

So far as the second factor is concerned, the view of Weinlig (5) can be considered in harmony with that of Hunziker. Weinlig believes that the effects of pasteurization are to be explained by a diminution in the concentration of the skin or membrane of albuminous colloid around the fat globules, which is accompanied by a change in its composition and tenacity. It is difficult to secure experimental data to support or deny this view. Weinlig offers none in support of it. Palmer and Samuelsson (10) found that the gold number of the crude "membrane" material is greatly decreased (i.e. suspensoid stabilizing property increased) by boiling. Similar, as yet unpublished (11) results have been obtained in this laboratory by pasteurizing solutions of the "membrane" material. It is not possible to say definitely that this finding has a bearing on Hunziker's and Weinlig's theories, but it does not at first sight seem to support them. A substance whose protective qualities are increased by heat would not be expected to lose tenacity.

Rahn's recent studies (9, 12, 13) of the cause of pasteurization effects on cream rising has led him to revert to an earlier explanation (14). According to this view it is the grouping of the fat globules into clusters in raw milk which is the chief factor in creaming giving rise to normal cream layers. Pasteurization effects are explained by the breaking up of these clusters. Babcock and Russell (15) found that cluster disintegration began at 140°F. and was complete at 150°F. Rahn believes that single globules have relatively little tendency to rise in raw milk. He observed that raw milk shows many clusters of fat globules and a chaos of movement of the single globules while in heated milk the clusters are lacking and the movement of the single fat globules regular and their rate of rise more rapid than for the single globules in raw milk. Rahn's (9) observations on the rate of rise of single fat globules in milk has also led him to conclude that the speed is too slow to account for the cream layers that form on raw milk in twelve to twenty-four hours.

In none of the studies so far reported has an attempt been made to examine experimentally the relation of the various plasma constituents to cream rising or to ascertain the effect of heat on these constituents in relation to the cream rising phenomenon.

The success attained in securing normal cream layers in milks prepared by addition of suitable amounts of very rich cream to skim milk suggested a mode of attack on this problem not heretofore undertaken. The present paper reports some of the outstanding features of the experiments.

Methods of procedure

The milk was fresh milk obtained from the University herd. Mixed milk from Holstein, Guernsey, Jersey, and Ayrshire cows was used unless the experiment called for high plasma solid (Jersey, Guernsey) or low plasma solid (Holstein) milk. The milk in most instances was standardized to contain 3.5 per cent fat as determined by the Babcock method. Pasteurization was effected by means of shotgun cans immersed in a vat of hot water.

Cream layers were determined in 200 cc. graduated cylinders. The height of the milk column in these cylinders was approximately 20 cm. Duplicate cylinders were placed at 0° and at 10°C. using a fireless cooker type of thermostat and melting ice for the low temperature and a vat of running tap water or an ice-cooled refrigerator for the higher temperature.

The percentage of fat in the skim milk layers was determined by analysis after drawing off the skim milk through a small hole near the base of the cylinders. From these data and the fat content of the whole milk the approximate concentration of fat in the cream was calculated by the formula*

$$C = \frac{100\,x - (100 - y)\,z}{y}$$

Where C = percentage of fat in the cream layer
 x = percentage of fat in the milk
 y = percentage of cream by volume
 z = percentage of fat in skim layer

Specific gravity was determined by the lactometer or Westphal balance. Solids-not-fat were calculated using the tables of

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*Author's note: The formula used for this calculation which was published in the first paper of this series, January, 1926, was in error. The formula as given above is correct.

Shaw and Eckles (16). Hydrogen ion concentrations were determined potentiometrically using the Bailey electrode, Leeds and Northrup Type K potentiometer, and normal calomel cell.

Casein was prepared from separated milk by the grain curd method (17) and purified by leaching for several days in frequent changes of distilled water adjusted to pH = 4.8. This casein was pressed dry, broken up into small pieces and used in the moist condition for the preparation of casein milks.

The casein milks were colloidal dispersions of calcium caseinate prepared by grinding the moist casein with suitable quantities of $CaCO_3$ and $Ca_3(PO_4)_2$ until a concentrated colloidal solution of calcium caseinate resulted. For example, in one experiment the casein from 155 pounds of skim milk was ground with 75 grams of $CaCO_3$ and 25 grams of $Ca_3(PO_4)_2$ and small amounts of water until a thick creamy paste resulted. This was diluted gradually with water with continued grinding until a moderately thin fluid resulted. Grinding was effected either by a colloid mill, or by a burr grinder. Excess $CaCO_3$ and $Ca_3(PO_4)_2$ were removed by a DeLaval No. 105 Clarifier. Casein concentrations were based on a nitrogen determination. In most cases a milk containing 9 to 10 per cent casein was used for the preparation of the various casein milks described below, suitable dilutions being made with water, lactose solution, cream, etc., to give products of the desired composition.

Whey was prepared by two methods, rennin and acid. Separator skim milk from mixed milk or from such breeds as it was desired to use to vary the concentration of whey proteins was treated with rennet extract or phosphoric acid. In the former clotting was allowed to take place at 32°C. and the curd cut and allowed to shrink at this temperature with very little agitation. The whey was drained off, clarified and analyzed for nitrogen by the Kjeldahl method. For the acid whey, casein was removed by the grain-curd method, the whey clarified and then neutralized to pH = 6.5 (determined potentiometrically) using a 5 per cent NaOH solution. After further clarification the whey was analyzed for nitrogen.

In certain experiments soluble salts of skim milk were removed

by dialysis. This was effected by placing the milk in collodion bags prepared in 200 cc. test tubes. These bags were prepared in the usual manner and then immersed in 1 per cent gelatin solution for fifteen minutes, followed by treatment with 2 per cent formaldehyde solution for the same length of time. The bags were then washed thoroughly with distilled water and kept immersed in water until used. The milk was allowed to dialyze in these bags under pressure, each bag being filled as full as possible and tied firmly with a rubber band. About 30 such bags were allowed to sink in cold distilled water placed in 5-gallon stone jars kept in the dairy refrigerator at 5° to 8°C. Dialysis was continued for twenty-four to thirty-six hours with several changes of water.

Experimental results

a. *Effect of pasteurizing only the skim milk or cream.* In order to ascertain the general relation of the plasma constituents to the effects of pasteurization on creaming, experiments were carried out first in which the plasma phase only was pasteurized and then used as the source of skim milk for the standardized product prepared from skim milk and cream. Several such experiments were conducted, the results of which are summarized in table 1. The data show that in most cases just as detrimental effects and in some cases much greater effects on cream rising are secured by pasteurizing the plasma phase of milk alone as when the whole milk is pasteurized. More striking results were secured in the first three experiments than in the later ones. The explanation is not clear. It may be stated, however, that the first three were a part of experiments carried out in 1922 and the others a year later. In spite of the variations in the extent of the destruction of cream rising ability, the data certainly point strongly to the conclusion that it is the plasma phase of milk which is affected chiefly in pasteurization and not the fat globules or substances associated with them through adsorption or other physico-chemical mechanism.

It is appreciated that these experiments call for others in which the cream only is pasteurized before mixing with the raw milk.

TABLE 1

Influence of pasteurizing the plasma on the creaming of raw milk

EXPERI- MENT NUMBER	DESCRIPTION OF MILK	TEMPERA- TURE OF PASTEURI- ZATION	TEMPERA- TURE OF SKIM WHEN ADDING CREAM	VOLUME CREAM AFTER TWENTY- FOUR HOURS AT 0°C.
		°C.	°C.	per cent
1	Raw, whole*...................		32	13.5
	Raw skim + raw cream............		23	13.3
	Pasteurized skim + raw cream.......	62	62	6.0
	Pasteurized skim + raw cream.......	67	67	2.0
2	Raw, whole....................		23	13.8
	Raw skim + raw cream............		23	13.8
	Pasteurized skim + raw cream.......	62	23	1.0
	Pasteurized skim + raw cream.......	62	23	1.8
	Pasteurized skim + raw cream.......	67	23	0.5
	Pasteurized skim + raw cream.......	67	67	0.8
3	Raw, whole....................		23	13.5
	Raw skim + raw cream............		23	12.9
	Pasteurized skim + raw cream.......	63	23	4.4
4	Raw skim + raw cream............		23	14.0
	(Raw skim + raw cream) pasteurized..	63	23	15.0
	Pasteurized skim + raw cream.......	63	23	9.0
5	Raw skim + raw cream............		23	15.0
	(Raw skim + raw cream) pasteurized....	63	23	9.0
	Pasteurized skim and raw cream......	63	23	11.0
6	Raw Holstein whole...............		23	11.5
	Raw Holstein skim + raw cream......		23	10.0
	Pasteurized Holstein...............	63	23	9.0
	(Holstein skim and cream) pasteurized.	63	23	5.5
	Holstein skim (pasteurized) + raw cream	63	23	8.5
7	Raw Jersey skim + raw cream.......		23	13.8
	(Jersey skim + cream) pasteurized....	63	23	9.2
	Jersey skim (pasteurized) + raw cream..	63	23	11.0

* Experiments 1 to 5 were conducted with mixed milk.

Experiments of this nature were not carried out chiefly because of the difficulties involved in pasteurizing small lots of high fat cream so as to avoid rendering the fat, and at the same time produce effects due to heat alone. The problem was therefore approached indirectly, namely, by using cream from pasteurized milk.

TABLE 2

Relative effect on cream rising of pasteurizing only the plasma or cream *

EXPERI-MENT NUMBER	DESCRIPTION OF MILK	VOLUME CREAM AFTER TWENTY-FOUR HOURS AT 0°C.	FAT IN SKIM	APPROXI-MATE FAT IN CREAM
		per cent	*per cent*	*per cent*
1	Raw, whole............................	16.0	0.50	19.3
	Raw skim + raw cream...............	13.5	0.55	22.4
	Pasteurized whole....................	10.5	1.55	20.2
	(Raw skim + raw cream) pasteurized..	8.5	1.58	24.3
	Raw skim + cream from pasteurized whole..............................	14.0	0.70	20.7
	Skim from pasteurized whole + raw cream...............................	10.0	1.73	19.5
2	Raw, whole............................	14.3	0.55	21.5
	Raw skim + raw cream...............	14.3	0.55	21.5
	Pasteurized, whole...................	11.0	1.28	21.5
	(Raw skim + raw cream) pasteurized..	9.3	1.70	21.1
	Raw skim + cream from pasteurized whole..............................	14.0	0.50	21.8
	Skim from pasteurized whole + raw cream...............................	5.3	2.20	26.9
	Pasteurized skim + raw cream........	6.0	2.18	24.2

* We are greatly indebted to Prof. W. B. Combs, Division of Dairy Husbandry, University of Minnesota for assistance in carrying out these experiments.

Two experiments of this kind are shown in table 2. In both experiments pasteurization was at 63°C. for thirty minutes in shotgun cans. In the first experiment the raw cream used for standardization contained 62.5 per cent fat, and the cream from the pasteurized milk contained 56.75 per cent fat. In the second experiment the raw and pasteurized cream tested 63.25 and 58.25 per cent fat, respectively.

The results of the two experiments were uniform in showing that the use of pasteurized cream in standardizing a synthetic whole milk is not detrimental to the creaming if the skim milk has not been pasteurized.   In other words the data demonstrate conclusively that it is the milk plasma and not the fat globules that are affected by heat so as to interfere with cream rising.

A careful study was made of the extent of fat globule clustering in connection with the second experiment of this group.   Observations were made on the freshly prepared samples at the time they were set aside for creaming, and also on the cream layers and remixed samples after the twenty-four-hour creaming period. The method employed was to prepare a hanging drop and observe the fat globules as they flowed past the field of observation. Flowing was induced by capillary attraction between the edge of the concave depression in the slide and the cover slip carrying the hanging drop.   No essential change in the extent of fat globule clustering of a single sample could be noticed after twenty-four hours standing in ice water.   The cream and remixed milk were also essentially the same except that more large fat globules were observed in the cream than in the mixed milk.

The most important observation made was the complete lack of agreement between the creaming phenomena and clustering. For example, of the three samples showing good cream volumes and exhaustive creaming, namely, those in which the whole milk or skim milk was raw, there were no clusters whatever in the two samples prepared from skim milk and separated cream (either raw or pasteurized), while the raw untreated milk showed considerable clustering, it being estimated that one-third of the fat globules existed in pairs or cluster of three or more globules.   A further lack of relationship between clustering and creaming was observed for the pasteurized samples, all of which showed some clustering, the maximum amount appearing in the sample which had the poorest cream layer.

These results are wholly irreconcilable with those theories which attempt to explain creaming variations by means of fat globule clustering or its absence.

b. *Relative influence of plasma colloids and plasma crystalloids*

on cream rising. Milk plasma is a mixture of various colloids and crystalloids. It is not to be expected that these two groups of substances exert a similar influence on cream rising of raw or pasteurized milk. However, the specific gravity of plasma is determined largely by the mineral salts and lactose in true solution so that it is a matter of speculation whether a synthetic milk consisting of a mixture of high test cream and plasma colloids could show normal cream rising properties. Moreover, the

TABLE 3

Cream rising of raw and pasteurized milk made from cream and dialyzed plasma

EXPERIMENT NUMBER	DESCRIPTION OF MILK	pH	SPECIFIC GRAVITY AT 15.5 °C.	CREAM VOLUME AFTER TWENTY-FOUR HOURS AT 0°C.	FAT IN WHOLE MILK	FAT IN SKIM LAYER	APPROXIMATE FAT IN CREAM LAYER
				per cent	*per cent*	*per cent*	*per cent*
1	(Natural skim + cream) raw........	6.48		10.3	3.6	2.1	16.7
	(Natural skim + cream) pasteurized.	6.59		12.8	3.6	1.7	16.6
	(Dialyzed skim + cream) raw.......	6.91	1.010	15.0	3.6	2.1	10.8
	(Dialyzed skim + cream) pasteurized.	6.84	1.016	10.0	3.6	2.3	15.3
2	(Natural skim + cream) raw........	6.49		15.0	3.9	1.0	20.3
	(Natural skim + cream) pasteurized.	6.32		9.0	3.9	2.2	21.1
	(Dialyzed skim + cream) raw.......	6.64	1.029	15.0	3.9	1.0	20.3
	(Dialyzed skim + cream) pasteurized	6.75	1.031	8.0	3.9	2.9	15.4
	Dialyzed skim (pasteurized) + raw cream...........................	6.58	1.034	14.0	4.7	2.7	17.0

influence of mineral salts on both hydrophilic and hydrophobic colloids (especially the latter) is so great that very interesting possibilities regarding creaming phenomena are suggested by a determination of the effect of removing the crystalloids from milk plasma.

Two experiments of this nature are reported in table 3. The results are to be looked upon as approximations only so far as removing the mineral salts and lactose and other substances in

true solution are concerned. In the first place the dialyzed plasma (skim milk) can be said to have had its crystalloid content merely reduced very greatly. This is shown by the low specific gravity of the milk in experiment 1. Furthermore, the cream used for standardizing the fat content of the milk contained milk crystalloids although in relatively greatly reduced quantity. The cream used for experiments 1 and 2 tested 41 and 51 per cent fat, respectively.

The nearly normal specific gravity of the milk in experiment 2 was brought about by the addition of lactose to the dialyzed milk in quantity sufficient to restore the original lactose content. Approximately 5 grams of lactose were added to each 100 cc. of dialyzed plasma. The purpose was to determine the relative influence of the lactose and soluble mineral salts on the cream rising phenomena.

The conditions surrounding these two experiments were such that the rise of fat was very much delayed resulting in skim milk layers of relatively high fat content. It is to be noted, however, that the removal of either the mineral salts or the mineral salts and lactose in large measure by dialysis had no detrimental effect on the cream rising of the raw milk. The influence of pasteurization was also essentially the same for the unaltered and dialyzed milk.

An interesting fact brought out in this experiment is that cream volumes may be very good in spite of a high fat content in the skim layer. The approximate fat content in the cream layer is also surprisingly high in these cases, and emphasizes the part that packing plays in determining the volume of cream rising on a given volume of milk. The line of demarkation between the cream and skim milk layers, i.e., the true "cream line" was not very distinct in these experiments. This result was noted particularly in the case of the raw dialyzed plasma plus cream in experiment 1.

Although these two experiments can not be regarded as giving conclusive proof of the relative importance of the crystalloids and colloids of milk plasma in cream rising, the results certainly point very strongly to the conclusion that the raw milk creaming

phenomena and pasteurization effects are governed chiefly if not entirely by the plasma colloids and that the crystalloids play little if any part in cream rising problems.

TABLE 4

Physical and chemical properties of raw and pasteurized calcium caseinate milk

EXPERIMENT NUMBER	SOURCE OF FAT	TREATMENT	CASEIN	SPECIFIC GRAVITY AT 15.5°C.	pH	CREAM VOLUME		FAT IN SKIM		APPROXIMATE FAT IN CREAM	
						At 0°C.	At 12°C.	At 0°C.	At 12°C.	At 0°C.	At 12°C.
			per cent			per cent	per cent	per cent	per cent	per cent	per cent
1	Holstein	Raw	2.39	1.0075	6.77	3.0	4.5	2.97	2.47	20.6	26.0
		Pasteurized	2.39	1.0075	6.71	2.0	3.8	2.97	2.40	24.5	31.3
	Jersey-Guernsey	Raw	2.39	1.007	6.78	5.9	8.4	2.25	1.65	23.4	23.7
		Pasteurized	2.39	1.007	6.75	2.7	5.9	2.57	1.57	37.0	34.4
2	Holstein	Raw	2.93	1.0076	6.78	3.5	6.0	3.02	2.37	17.0	21.2
		Pasteurized	2.93	1.0076	6.73	2.6	5.1	2.90	2.25	26.0	26.8
	Jersey-Guernsey	Raw	2.93	1.0079	6.75	11.3	10.4	1.05	0.60	22.7	25.5
		Pasteurized	2.93	1.0079	6.72	3.5	5.3	2.40	1.52	33.8	38.9
3	Holstein	Raw	2.39	1.0239	6.12	2.5	6.8	2.80	2.63	29.8	15.4
		Pasteurized	2.39	1.0262	6.24	2.0	3.0	3.32	3.22	12.2	12.6
	Jersey-Guernsey	Raw	2.93	1.0262	6.02	9.0	5.5	2.48	2.58	14.9	21.1
		Pasteurized	2.93	1.0282	6.19	2.0	2.6	3.36	3.00	15.2	26.1
4	Holstein	Raw	2.39	1.0256	6.54	8.0	7.0	2.55	2.30	15.7	20.9
		Pasteurized	2.39	1.0280	6.54	4.0	6.3	3.00	2.27	18.0	23.4
	Jersey-Guernsey	Raw	2.93	1.0294	6.50	7.3	5.3	2.60	2.37	16.3	25.6
		Pasteurized	2.93	1.0314	6.50	5.8	7.5	2.65	1.90	19.0	24.6
5	Holstein	Raw	2.39	1.0278	6.59	6.0		2.35		17.4	
		Pasteurized	2.39	1.0298	6.59	2.0	2.0	2.90	2.60	23.0	32.5
	Jersey-Guernsey	Raw	2.93	1.0287	6.59	10.5	7.5	0.50		22.4	
		Pasteurized	2.93	1.0303	6.59	6.5	5.7	0.60		34.4	

c. Calcium caseinate as a factor in cream rising. The experiments reported in the preceding sections at once raise the question

as to which colloidal constituents of milk plasma are affected by heat so as to interfere with cream rising.   As already pointed out Hunziker (2) holds that a coagulation of lactalbumin is probably responsible, in part, for abnormal cream rising of pasteurized milk.   Casein, however, is by far the most abundant of the plasma colloids.   The first experiments were therefore carried out for the purpose of determining its relation to the cream rising phenomena.

Five experiments in all were conducted using calcium caseinate milk.   The data are summarized in table 4.   Inasmuch as the experiments differed slightly in certain details, a brief description of the salient features of each seems advisable.

The preparation of the calcium caseinate dispersion was practically the same for each experiment.   The method employed has already been described.   In experiment 1 the casein concentration of 2.36 per cent is intended to represent Holstein milk.   The concentration 2.93 per cent used in experiment 2 represents Jersey milk.   In the remaining experiments milk was prepared representing both Holstein and Jersey.   The concentrations selected are based on the data of Eckles and Shaw (18) on the relation of breed to composition of milk.

All the experiments were alike in that the cream used for standardizing the artificial whole milk was secured from both Holstein and Jersey-Guernsey whole milk.   In experiments 1 and 2 small and large fat globule cream was added to the single plasma prepared for each experiment.   In the remaining experiments Holstein cream was added only to Holstein plasma and Jersey-Guernsey cream to Jersey-Guernsey plasma.   The fat content of the creams used for the synthetic whole milk in the various experiments was as follows:

EXPERIMENT	HOLSTEIN	JERSEY-GUERNSEY
1	51.5	56.5
2	51.5	56.6
3	58.0	63.0
4	54.0	60.0
5	52.0	60.0

It was intended that each lot of synthetic whole milk should have 3.5 per cent fat.   The results did not deviate more than 0.1 per cent from this except in experiment 5 where the Jersey milk tested only 2.8 per cent fat and the Holstein 3.3 per cent.

In experiments 1 and 2 no attempt was made to control the specific gravity of the synthetic milk which accounts for the abnormally low values reported.   In experiments 3, 4, and 5, however, the calcium caseinate was diluted with sufficient 10 per cent lactose solution as well as cream to give the milks a sugar content of 5 per cent for the Jersey milks and 4.65 per cent for the Holstein milks.   This increased the specific gravity to values which approached more nearly that of normal milk.

The pH of the milk was not controlled in the first three experiments except in so far as the calcium caseinate was prepared as nearly neutral as possible.   This accounts for the high pH of the milks in experiments 1 and 2 and the low pH of experiment 3. In experiments 4 and 5, however, the pH value of the milks was increased with a 10 per cent Na_2HPO_4 solution to approximately 6.5 to 6.6.   This effectively overcame a slight tendency to coagulate which was noticed in the pasteurization of the milk in experiment 3.

The pasteurization procedure was the same for all experiments. When the milk in the shot gun cans had reached 63°C., the gentle agitation was discontinued throughout the holding time of thirty minutes except when temperature readings were taken at five-minute intervals.

The results were essentially the same in each experiment regardless of the pH, specific gravity, relative size of fat globules or variation in casein content as representing Holstein or Jersey milk.   It was surprising that the specific gravity had so little effect.   Since the variation of this property was brought about by lactose, it seems evident that the sugar content of milk has no influence on cream rising.

One outstanding feature of the results is the uniformly poor cream layers on the raw milk and the uniformly detrimental effect of pasteurization.   The data show, of course, that one primary reason for the poor cream layers is the fact that in a

great majority of cases only a very small proportion of the fat rose on the casein milk. This can not be the sole explanation, however, inasmuch as very good cream layers were obtained in the dialysis experiments (table 3) in spite of a high concentration of fat in the skim milk layer.

It seems fairly obvious that calcium caseinate although the most prominent colloid in the plasma has relatively little to do with either promoting the rise of the fat globules or increasing the volume which they occupy as cream. It should be noted, however, that there is in general a duplication of the results secured in the previous study (1) in that pasteurization results in a less exhaustive creaming and a closer packing of the fat globules in the cream layer. It can not be decided definitely with the data now at hand whether this is an effect of pasteurization on calcium caseinate or to some other factor, but the indications point strongly to calcium caseinate as the constituent affected.

Another outstanding and in fact remarkable feature of these experiments is the increase in cream layers at 12°C. in comparison with 0°C. This result alone points conclusively to the fact that calcium caseinate is not the colloidal constituent of milk plasma which promotes normal cream rising. Natural milk always has a lesser cream layer at high temperatures than when kept cold.

A further fact not brought out in table 4 pointing to the same conclusion regarding the rôle of calcium caseinate in cream rising is that the line of demarkation between the cream and skim milk was very indistinct in many of these experiments especially in the cylinders containing pasteurized products. In many instances it was necessary to determine the cream layers by holding the cylinders before a strong light.

The experience with calcium caseinate suggests that if plasma colloids are responsible in the main for normal cream rising, it is the true hydrophils, lactalbumin and globulin that are concerned. The experiments reported in the next section throw light on this relationship.

d. *Whey colloids as factors in cream rising.* The experiments

to determine the influence of the whey colloids (chiefly lactalbumin and globulin) on creaming were carried out in essentially the same manner as the calcium caseinate experiments. The whey studies, however, naturally fall into two groups. In the first group, the results of which are presented in table 5, rennet whey of Holstein or Jersey-Guernsey or mixed origin was com-

TABLE 5

Whey colloids as a factor in cream rising on raw or pasteurized milk

EXPERIMENT NUMBER	SOURCE OF CREAM	PROTEIN CONTENT OF WHEY	SPECIFIC GRAVITY AT 15.5°C.	pH	TEMPERATURE OF PASTEURIZATION	CREAM VOLUME		FAT IN SKIM LAYER		APPROXIMATE FAT CONTENT OF CREAM	
						At 0°C.	At 12°C.	At 0°C.	At 12°C.	At 0°C.	At 12°C.
		per cent			°C.	per cent	per cent	per cent	per cent	per cent	per cent
1	Holstein	0.96	1.0247	6.34	Raw	11.5	5.0	2.00	1.90	15.1	33.9
		0.96	1.0247	6.42	63	16.3	7.8	1.95	1.75	11.5	24.3
	Jersey-Guernsey	0.96	1.0250	6.36	Raw	17.0	7.0	1.57	1.32	7.0	18.3
		0.96	1.0250		63	20.0	9.0	1.55	1.27	6.3	14.9
2	Jersey-Guernsey	1.08	1.0264	6.53	Raw	14.0	8.9	0.57	1.40	21.5	13.8
		1.08	1.0264	6.45	63	14.3	12.5	0.25	0.32	23.1	25.7
	Holstein	1.08	1.0263	6.48	Raw	13.6	7.5	0.80	1.60	21.4	26.9
		1.08	1.0263	6.46	63	15.8	13.8	0.40	0.45	20.1	22.6
3	Mixed		1.0264	6.45	Raw	13.0		0.80		21.6	
			1.0269	6.52	63	18.8		1.40		7.3	
			1.0279	6.53	67	17.0		1.60		6.9	
			1.0279	6.53	63*	10.0		1.85		18.4	

* Whey only pasteurized.

bined with Holstein or Jersey-Guernsey or mixed cream in various combinations to determine the effect of two levels of whey protein in connection with large and small fat globules. In particular, however, the pH of the synthetic milk in the experiments was nearly identical with that of fresh natural milk.

The second group shown in table 6 compares the use of acid and rennet whey as a plasma for synthetic milk in cream rising

studies.   These milks differed from those in the first group by being somewhat acid in comparison with natural milk and therefore show in general the effect of increased acidity on cream rising.

In both groups of experiments pasteurization was carried out in shot gun cans.   The "milk" was gently agitated until the

TABLE 6

Whey colloids as factors in cream rising of raw or pasteurized milk of relatively low pH

EXPERIMENT NUMBER	KIND OF WHEY	PROTEIN CONTENT OF WHEY	SPECIFIC GRAVITY AT 15.5°C.	pH	TEMPERATURE OF PASTEURIZATION	CREAM VOLUME AT 0°C.	FAT IN SKIM LAYER	APPROXIMATE FAT CONTENT OF CREAM LAYER
		per cent			*°C.*	*per cent*	*per cent*	*per cent*
1	Rennet..............	1.10	1.0259	6.12	Raw	7.6	0.82	36.0
		1.10	1.0259	6.12	63	7.6	1.45	28.3
		1.10	1.0258	6.12	Raw	23.5	0.02	10.0
		1.10	1.0258	6.13	63	26.6	1.01	10.4
2	Rennet..............		1.0245	6.26	Raw	8.0	1.70	24.2
			1.0245	6.32	63	12.0	1.50	18.2
			1.0245	6.29	63*	5.8	2.45	20.7
3	Rennet..............		1.0245	6.10	Raw	6.0	2.70	16.0
			1.0245	6.20	63	12.0	1.50	18.2
4	Acid..............		1.0241	6.06	Raw	10.0		
			1.0232	6.27	63	12.0		
			1.0233	6.28	67	10.0		
			1.0236	6.28	63*	10.0		

* Whey only pasteurized.

desired temperature of pasteurization was reached.   There was also gentle agitation at five minute intervals during the 30-minute pasteurization period.   In all cases the "milk" was cooled to 26°C. before placing in the creaming cylinders or mixing with cream in case the whey only was pasteurized.

Before pointing out the important features of the results of these experiments, it should be stated that pasteurization pro-

duced no visible effects on any of the whey milks. This was also true for the tests in which the whey alone was pasteurized before mixing with the cream.

In many respects the results with the whey milks were contrary to those obtained with natural or casein milks. It will be noted first that when the pH was normal the raw milk gave excellent cream layers and that these increased somewhat in proportion to the protein content of the whey. However, contrary to any previous result pasteurization gave a marked *increase* to the cream layer, even when the pasteurization temperature was sufficiently high to practically destroy cream rising on natural or casein milk. Similar but less marked increases in cream layers occurred when the more acid whey milks were pasteurized.

The effect of heating the whey milks on the rise of fat and its distribution between cream and skim milk layer is particularly interesting. The data show that with whey milks of normal pH (table 5) the result was a *more* exhaustive creaming and a *looser* packing of the fat in the cream. This is entirely opposite to the effect of heat on natural or casein milks which show a *less* exhaustive creaming and a *closer* packing of the fat in the cream. When the whey milks were somewhat acid, the results were not regular in these respects showing that other factors were operating which require further study for elucidation.

The object of comparing rennet and acid wheys was to determine whether the rennet enzymes remaining in the whey might be acting on the traces of casein added with the cream and thus be a factor in some of the surprising results secured. Unfortunately the acid and rennet wheys which are comparable are each somewhat acid in comparison with normal milk. There is nothing in the results, however, to indicate an effect exerted by rennet enzymes.

DISCUSSION

The results of this series of experiments give a particularly close insight into the fundamental factors operating in cream rising on raw and pasteurized milk. They show conclusively that plasma colloids are the chief, if not the only constituents of

milk that exert an influence in cream rising.   It appears that the difference between the specific gravity of fat and of milk plasma as represented by lactose and salts has very little to do with the volume of the cream layer although the exhaustiveness of cream rising is much affected.   However, it is also clear that exhaustiveness of creaming under uniform conditions is influenced so greatly by other factors that specific gravity differences are readily and often almost completely overcome.

Our study of the comparative effects of the two important colloids of milk, calcium caseinate and lactalbumin (as represented by whey) has led to surprising and unexpected results. The most prominent colloid of milk, calcium caseinate, is shown to be a very poor promoter of satisfactory cream rising or ample cream layers.   When heated this colloid is even more detrimental to both the rise of cream and to a sufficient separation of the fat particles in the cream to give a deep cream layer. These results lead one to suspect that when an exhaustive cream rise occurs accompanied by a cream layer of satisfactory depth, it does so in spite of the presence of calcium caseinate; and when the detrimental effects of pasteurization are manifested, calcium caseinate is chiefly to blame for the result.

On the other hand the whey colloids are clearly promoters of satisfactory cream layers, and more surprising still their effectiveness is increased by pasteurization both with respect to more exhaustive creaming as well as cream layers of greater volume. Some of the older explanations of pasteurization effects on creaming thus become entirely untenable.   The idea that a lactalbumin coagulation is responsible for the unsatisfactory cream layers on pasteurized milk is not substantiated.   On the contrary whatever effect pasteurization exerts on lactalbumin is a gain rather than a hindrance to cream rising and cream layers.

At first sight it seems very surprising that calcium caseinate and lactalbumin are found to exert an opposing effect on cream rising phenomena.   The exact cause of this requires further experimental study.   Nevertheless the suggestion may be permitted that calcium caseinate and lactalbumin do not belong to the same class of colloids.   As one of us (19) has recently

pointed out calcium caseinate has many of the properties of a suspensoid (hydrophobe) although also possessing important properties of an emulsoid (hydrophile). It is thus evident that the opposing effects of calcium caseinate and whey colloids on cream rising are in some way related to differences in the character of the colloids. It should be emphasized, however, that the questions involved in cream rising do not include that of emulsion stability. While there may be differences between calcium caseinate and whey proteins so far as emulsion stabilization is concerned both kinds of proteins are to be considered as excellent emulsifiers.[1] The creaming phenomena with which this study has dealt are cream rising and volume of cream layer. In general our results point to the conclusion that both exhaustiveness of rise of fat and greater volume of cream are promoted by the more truly hydrophillic colloids and depressed by colloids with hydrophobic properties. Pasteurization at 63°C. for thirty minutes increases the effectiveness of the former but decreases still further the effectiveness of the latter. When the emulsion contains both classes of colloids, it may be expected that the net result will be determined largely by the colloid present in greater concentration. This supposition in fact offers a plausible explanation of the general detrimental effect of pasteurization on creaming of whole milk.

It is not possible to reconcile the results of any phase of this study with those theories which seek to explain cream rising changes on the basis of fat globule clustering. Rahn (9, 12, 13) has recently emphasized these theories. Our studies show conclusively that fat clustering is not even uniformly coincident with either satisfactory cream volumes or exhaustive creaming and is not to be regarded as a primary cause of cream rising changes. The data presented in tables 1 and 2 in particular make it exceedingly difficult to believe that the mode of distribution of the fat globules could have played any significant part in the results. In fact as already pointed out in the presentation

[1] A comparison of the gold number of calcium caseinate and lactalbumin by Mr. Johnson (11) in this laboratory gives calcium caseinate a protective value (reciprocal of gold number) of 83 and of lactalbumin of 20.

of the data of the experiment shown in table 2, a careful examination of the wholly standardized milks of experiment 2, i.e., all those in which skim milk was mixed with either raw or pasteurized cream, failed to disclose clustering of the fat globules in any case; yet there was marked impairment of cream rising whenever the skim milk or the mixture of skim and cream had been pasteurized in spite of the fact that clustering was not entirely destroyed.

This experiment also fails to support Weinlig's (5) theory that pasteurization effects are to be explained by a change in the properties of the membrane surrounding the fat globules.   While our data do not refute the assertion that some change occurs, it is obvious that it bears no relation to creaming.

CONCLUSIONS

1. The exhaustiveness of cream rising and the volume of the cream layer on cow's milk are determined largely by the plasma colloids.

2. Differences in density of fat and plasma do not determine the volume of cream and will not assure exhaustive rise of fat.

3. The calcium caseinate of cow's milk *hinders* cream rising and *does not promote* satisfactory cream volumes.   These effects are increased by those pasteurization procedures which depress the cream rising of natural milk and give rise to smaller cream volumes.

4. The whey colloids of cow's milk *promote* both cream rising and satisfactory cream volumes.   These effects are increased by the pasteurization procedures that give rise to unsatisfactory creaming of natural milk.

5. The detrimental effects of pasteurization on cream rising phenomena of natural milk are to be explained by the effect of heat on the predominating plasma colloid, calcium caseinate.

6. Changes in cream rising and cream layers on natural milk, especially those brought about by pasteurization, can not be explained solely by fat globule clustering or dispersion.

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SOME OBSERVATIONS ON THE FREEZING POINT OF MILK*

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The cryoscopic method has been advanced by Hortvet (1) and others for the detection and determination of added water in milk. In the published work no mention has been made of the possible effect of dissolved substances in the water with which the milk was adulterated. Since the depression of the freezing point is dependent upon the number of dissolved particles in solution, it is important to know to what extent these dissolved substances would effect the cryoscopic index of milk.

Another factor that has not been sufficiently investigated is the effect of seasonal variation on the freezing point. Since the various constituents of milk vary to some degree with the season, it would be quite possible to expect a slight variation in freezing point due to seasonal conditions. Stoecklin (2) indicates that there is no seasonal variation.

This investigation was planned to include the two factors: (*a*) effect of dissolved substances, and (*b*) seasonal variation.

Hortvet (1) in an article on "The cryoscopy of milk" based on his report as Referee on Dairy Products at Convention of The Association of Official Agricultural Chemists, Washington, D. C., 1920 has made an exhaustive search of the literature on the subject. About twenty-five references are mentioned. These generally agree that the freezing point of normal herd milk falls within rather narrow limits, an average being about −0.55°C. One of these investigators states "as a result of tests applied on 2500 samples in four years that under all conditions milk does not vary in freezing point outside the limits of −0.545° and −0.565°" (3). Hortvet (4) examined a large number of samples of known purity from individual cows and herds. These were adulterated with various percentages of water and the amount

determined by the cryoscopic method. His work shows that when the original freezing point is known, the amount of water added may be detected within 0.5 per cent. However, when −0.55° is taken as the freezing point, the added water was detected within less than 3 per cent.

Since the above mentioned report, several articles have been published (5, 6, 7) dealing largely with apparatus, precautions, effect of acidity and disease on detection of added water by this method. However, no mention is made of the effect of solutions with dissolved materials and the detection of the added water by the cryoscope.

In this work, the standard Hortvet cryoscope was used. The thermometer was standardized by the Bureau of Standards Sugar Solution Method recommended by Hortvet (8). Readings were made with a telescopic thermometer reader. The stirring was accomplished with a mechanical stirring device with an up and down motion delivering a stroke about once per second. All samples of milk were iced as soon as received.

I. INORGANIC SALTS AND SUCROSE AS A FACTOR

Since the depression of the freezing point of a liquid depends upon the number of particles in solution in the liquid, it would seem to follow that the accuracy of this method when applied for the purpose of detecting watering in milk would depend upon whether the water added was pure or a hard water containing considerable amounts of the mineral salts in solution. In other words, would the salts dissolved in a hard water overcome to a certain extent the effect of the added water? If this were the case, it would be possible to add an appreciable amount of such a water without causing too great a change in the freezing point.

An examination of the waters of Iowa (9) show many waters of a considerable degree of hardness. Some run as high as 9000 parts per million of dissolved minerals while values ranging from 3000 to 6000 parts per million are not at all uncommon. In order to determine the effect of such waters when added to milk on the freezing point of the milk, a water containing 1300 parts per million was added to the extent of 10 per cent. This water

when frozen against distilled water gave a freezing point which averaged consistently 0.02° lower. When distilled water was diluted with 10 per cent of this water, the freezing point was consistently 0.002°C. lower. Milk, when diluted with 10 per cent of this water, gave the same freezing point as milk diluted with 10 per cent distilled water. Table 1 shows these results.

From these data, we may conclude that waters with considerable dissolved minerals may be added to milk and that the salts present will not be effective towards changing the freezing point as determined by the cryoscope.

TABLE 1

ORIGINAL FREEZING POINTS OF FRESH MILK	FREEZING POINTS OF MILK 10 PER CENT VOLUME OF DISTILLED H_2O	FREEZING POINTS OF MILK DILUTED WITH 10 PER CENT VOLUME WATER
−0.543	−0.486	−0.48
−0.555	−0.495	−0.495
−0.551	−0.495	−0.495
−0.5507	−0.518	−0.5185
−0.554	−0.496	−0.496
	−0.523	−0.523*
−0.559	−0.4955	−0.496
−0.560	−0.500	−0.5005

* Four determinations were made on this sample without any variation of the freezing point.

The next step was the determination of the concentration of dissolved material necessary to become effective in varying the freezing point of milk and the relative effectiveness of different concentrations in overcoming the effect of added water. Several different solutions were used in the investigation of which magnesium chloride and sucrose solutions will be used to illustrate the results.

The various concentrations of solutions were based on calculations from Winter's formula,

$$W = \frac{(t - t')\,100}{t}$$

where W is the percentage of added water, t is the freezing point of original milk and t' is the freezing point of the adulterated

sample. Not knowing what the freezing points of the original milks would be, $-0.55°C$. was taken arbitrarily for the value of t. With this as an arbitrary value for t, if $t - t'$ were 0.0275, then W equals 5, if $t - t'$ were 0.055, then W becomes 10, etc. When the various concentrations of salts in solution were frozen and the depressions inserted in the formula for $t - t'$, W was calculated and called the "water equivalent" because it was assumed when these solutions were added to milk that the

TABLE 2

Effect of added magnesium chloride solutions

(1)	(2)	(3)	(4)	(5)	(6)
MOLAR CONCENTRATION	EXPERIMENTAL FREEZING POINTS DEPRESSION OF SOLUTION	WATER EQUIVALENT BY $W = \dfrac{(t - t')\,100}{t}$	CALCULATED WATER EQUIVALENT OF 10 PER CENT VOLUME ADDED TO MILK	MOLAR CONCENTRATION OF $MgCl_2 \cdot 6H_2O$ in MILK DILUTED 10 PER CENT	APPARENT PER CENT WATER PRESENT $W = \dfrac{(t - t')\,100}{t}$
				0.0000 (Distilled H_2O)	10.1
0.00985	0.0417	7.2	0.72	0.000985	10.1
0.0295				0.00295	8.2
0.0920	0.240	43.6	4.36	0.00492	5.9
0.0690	0.330	60.0	6.00	0.0069	4.5
0.0887	0.419	76.2	7.62	0.00887	3.14
0.108	0.513	93.2	9.32	0.0108	0.88
0.128	0.601	109.3	10.93	0.0128	−0.17
0.148	0.686	124.7	12.47	0.0148	−2.1
0.167	0.792	144.0	14.40	0.0167	−3.5
0.187	0.872	158.5	15.85	0.0187	−5.2

presence of the salt would cause a depression in the freezing point of the mixture which would compensate for the rise of freezing point caused by W amount of water added.

Assuming complete ionization for $MgCl_2 \cdot 6H_2O$, the following solutions were made up giving the various freezing point depressions found in column (2) of table 2. The different "water equivalents" as determined by

$$W = \frac{(t - t')\,100}{t}$$

where t is 0.55°, are found in column 3. The "water equivalent" values in column 6 were secured by diluting the milk with a 10 per cent volume of the magnesium chloride solutions. These dilutions were then frozen and the amount of water present was calculated by use of the Winter's formula. Figure 1 was plotted from these data.

In making figure 1 the data in column 5, table 2, was used for the ordinate while the values in column 6 were used for the abscissa. In calculating the values for column 6, the exact freez-

ing points of the original milk was substituted for t in Winter's formula. It should be noted that the smallest concentration of salt was not effective in lowering the higher freezing point of the adulterated milk since it froze at the same point as when 10 per cent distilled water was added. This concentration was equivalent to 1519 parts per million of dissolved magnesium chloride and should have counteracted the effect of 0.72 per cent of added water.
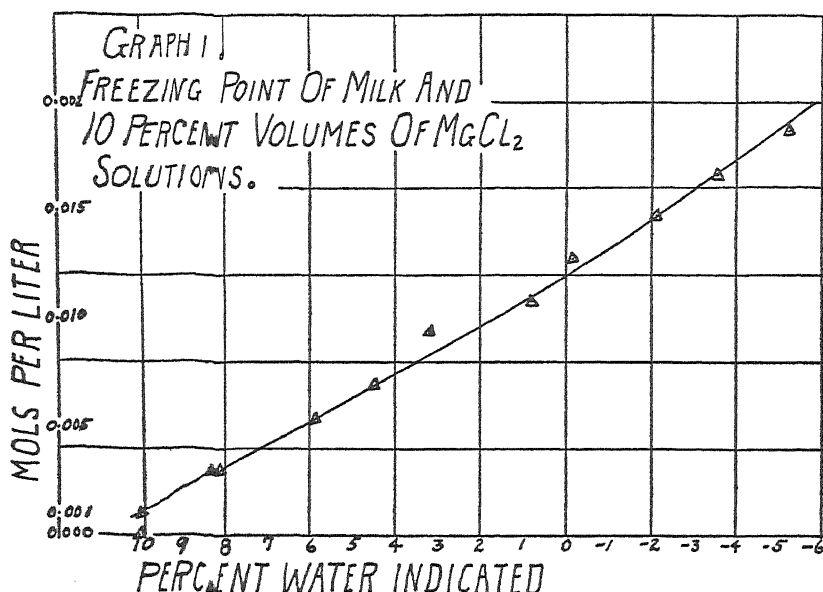
The next concentration of salt was effective inasmuch as it counteracted the effect of 1.9 per cent added water. This

concentration was equivalent to 3189 parts per million of dissolved material. A gradual lowering of freezing point continues until the salt is effective in overcoming completely the 10 per cent volume of water present from the solution. Additional concentrations lower the freezing point below that of the original milk and when calculated by the formula show a negative presence of water.

The largest concentration of salt should have been equivalent to 15.85 per cent added water according to column 4 and it

TABLE 3

Effect of added sucrose solutions

(1)	(2)	(3)	(4)	(5)	(6)
MOLAR CONCENTRATION	CALCULATED FREEZING POINTS DEPRESSION	CALCULATED EQUIVALENT OF H_2O $W = \dfrac{(t - t')\,100}{t}$	MOLAR CONCENTRATION OF SUCROSE SOLUTION IN MILK DILUTED 10 PER CENT	CALCULATED WATER EQUIVALENT OF 10 PER CENT VOLUME ADDED TO MILK	APPARENT PER CENT WATER PRESENT $W = \dfrac{(t - t')\,100}{t}$
	°C.				
			0.0000 (Distilled H_2O)		10.4
0.01479	0.0275	5	0.001479	0.5	10.4
0.0444	0.055	15	0.00444	1.5	9.2
0.0739	0.110	25	0.00739	2.5	7.6
0.1035	0.165	35	0.01035	3.5	5.2
0.1330	0.220	45	0.01330	4.5	4.5
0.1627	0.275	55	0.01627	5.5	3.0
0.1922	0.330	65	0.01922	6.5	2.6
0.2218	0.385	75	0.02218	7.5	1.7
0.2514	0.440	85	0.02514	8.5	1.25
0.2809	0.495	95	0.02809	9.5	−0.5

should be noted from column 6 that a negative depression equivalent to 5.2 per cent was secured, which when added to 10.1, the amount of water present, makes a total of 15.3 per cent water. This agrees quite well with the predictions.

It should be added here that the second concentration of milk and salt solution tasted decidedly salty.

Table 3 is similar to table 2 excepting that the freezing points depressions (column 2) of the various sucrose solutions were not obtained experimentally. These values were calculated on the

assumption that one molar weight of the sucrose in a liter solution would lower the freezing point 1.86°C. Using the calculated depressions in column 2, the equivalent water values found in column 3 were calculated by use of Winter's formula using 0.55 for t.

GRAPH II.
FREEZING POINT OF MILK
AND 10 PERCENT VOLUMES
OF SUGAR SOLUTIONS.

MOLS PER LITER

PERCENT WATER INDICATED
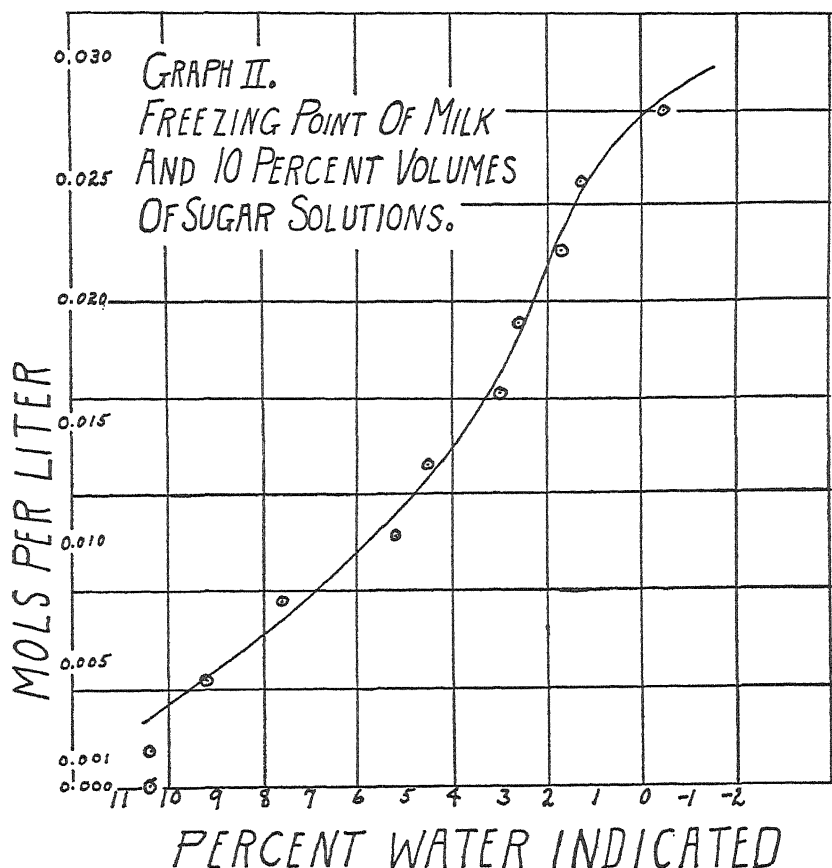
To make figure 2, the values in column 4, table 3, were used as the abscissa and those in column 6 as the ordinate. Again, it should be noted that the first concentration of sucrose was not effective in overcoming the effect of the water from the solution since it gave the same freezing point as when 10.4 per cent volume of distilled water was added. This concentration of sucrose is

equivalent to 5060 parts per million of dissolved material.   The next larger concentration is equivalent to 15,180 parts per

TABLE 4

DATE 1924	ORIGINAL FREEZING POINT OF MILK	FREEZING POINT OF MILK AND 10 PER CENT BY VOLUME OF DISTILLED WATER	CALCULATED PER CENT H_2O BY $W = \dfrac{(t - t')\,100}{t}$	CALCULATED PER CENT H_2O BY $W = \dfrac{(0.55 - t')\,100}{0.55}$	pH
Group A (Guernsey herd)					
4–26	−0.546	−0.489	10.4	10.3	
5–1	−0.544	−0.483	11.9	11.1	
5–6	−0.555	−0.495	11.2	10.9	
5–7	−0.553				6.604
5–8	−0.543	−0.486	10.5	10.3	6.689
5–20	−0.553	−0.491	11.2	11.2	6.532
5–21	−0.560				6.638
6–11	−0.5507				
6–12	−0.5448	−0.488	10.4	10.3	
6–13	−0.5527				6.531
6–15	−0.5695				6.276
Group B (2 Jerseys)					
6–24	−0.5507	−0.495	10.11	10.1	6.5
6–25	−0.554	−0.496	10.47	10.5	6.503
6–27	−0.559	−0.496	11.3	11.4	6.57
6–28	−0.561	−0.500	10.87	11.1	6.65
6–30	−0.557	−0.495	11.13	11.3	6.56
7–5	−0.555				6.53
7–7	−0.5586	−0.503	10.02	10.1	6.59
7–9	−0.572	−0.512	10.5	10.9	6.55
7–10	−0.562	−0.498	11.3	11.5	6.61
7–12	−0.563	−0.506	10.1	10.4	
7–14	−0.557	−0.500	10.2	10.3	
7–15	−0.563	−0.504	10.5	10.7	
7–24	−0.578	−0.517	10.6	11.1	
8–19	−0.572	−0.514	10.6	10.5	
8–21	−0.578	−0.516	10.7	11.2	
8–26	−0.578	−0.518	10.4	10.9	

million and by calculation should have overcome the effect of 1.5 per cent added water.   Experimentally, it succeeded in counteracting the effect of 1.2 per cent of added water.   Addition of

larger concentrations caused the freezing point to lower until that of the original was reached, successfully counteracting the effect of the 10.4 volume of the water added.

Explanation of these phenomena may be found in the adsorption theory. However, this is a subject for further investigation, which is being carried on at the present time.

II. SEASONAL VARIATION AS A FACTOR

Work was done on milk from two known sources and covering a period of four months from April 26 to August 26. Source

GRAPH III.
SHOWING EFFECT OF SEASONAL CHANGE
ON DEPRESSION OF FREEZING POINT.
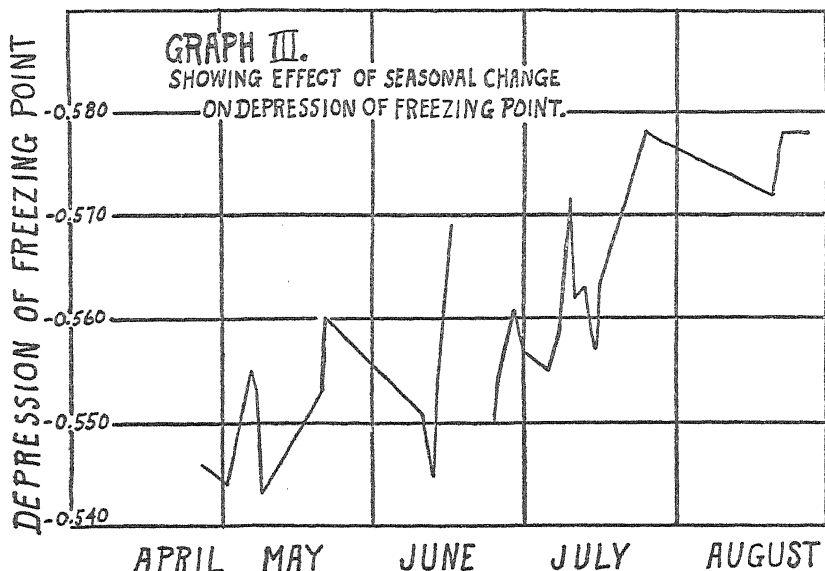
A in table 4 was a Guernsey herd and was used from April 26 to June 15. Source B was a herd of two Jerseys and was continued until August 26. Samples were packed in ice as soon as received. Table 4 is a record of these results and figure 3 is made from some of the data in this table.

Figure 3 was made by plotting the freezing points of the original milk as the ordinate against the time of season as abscissa. It seems to indicate rather clearly that there is a tendency for the freezing point to lower as the season advances.

There is a considerable fluctuation in the freezing points of the samples from group A but group B is much more consistent and shows a distinct tendency to lower as the season advances. pH values were determined on a number of the samples from both herds. An examination of the data in table 4 will show that there is no correlation between them and the lowering of the freezing point as the season progresses. An explanation for the seasonal change may be due to a change in food. This explanation disagrees in part with some of the data collected by Hortvet (1). In this paper the work of one investigator is summed up in the following generalization:

Milk, freshly drawn, from any variety cow, whether high or low breed, from whatever region of the country, from animals in stable or in pasture, whether poorly or substantially fed, whether drawn from a period near or remote from parturition, in winter or summer, morning or evening, whether the yield be scant or abundant, has a definite freezing point which varies but little around −0.550° although under various influences the chemical composition changes in enormous proportions.

Our data indicate that there is a consistent change in the freezing point during change of seasons.

III. CONCLUSIONS

The work on the factors affecting the freezing point of milk as determined by the cryoscopic method may be summed up as follows: (a) Equal volumes of distilled water and natural water of considerable hardness when added to milk give the same freezing point depression as detected by the cryoscopic method. (b) Magnesium chloride solutions containing at least 1519 parts per million may be added to milk without affecting the detection of the water in the solution by the cryoscopic method. Concentrations as great as 3189 parts per million counteract the effect of 1.9 per cent added. Greater concentrations counteract correspondingly greater quantities of water. (c) Concentrations of sucrose in solution as great as 5060 parts per million may be added to milk without affecting the detection of the water of the solution by the cryoscopic method; 15,180

parts per million counteracted the effect of 1.2 per cent added water. (*d*) There is a tendency for the freezing point of milk from the same herd to lower as the season advances from spring through the summer. (*e*) There is no correlation between the pH values and the freezing points of the milk during these seasons.

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A QUANTITATIVE FORM OF EXPRESSING PERSIST-ENCY OF MILK OR FAT SECRETION*

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It has been shown by work at this Station (1) that the secretion of milk and fat during the course of the lactation period rises for a time, reaches a maximum, and then gradually declines. It was pointed out that the rise in milk secretion becomes less and less as it approaches the maximum and the decline in this rise is exponential. After the maximum period of milk secretion, the curve of production declines and also follows an exponential law.

The exponential law, called by Lord Kelvin, the compound interest law which was found to express the change in the rate of milk secretion during the lactation period may be stated for our purpose as follows: When all other conditions are uniform the monthly milk or fat production during the lactation period after the maximum is passed, is a constant percentage of the pre-ceeding month's production.

This law holds true to a remarkable degree with non-pregnant cows, but in the case of pregnant cows there is a more rapid de-cline during the last four months of pregnancy (2). This factor as well as seasonal changes of environmental temperature, nu-trition, and management may cause slight changes in the rate of decline of milk and fat secreted (3).

The fact that the rate of increase and of decline of milk and fat secretion follows this important law of nature is of great value in the proper analysis of experimental results where milk and fat secretion is used as a criteria.

The purpose of this paper is to illustrate how this law may be employed in expressing, in a quantitative form, the persistency of milk or fat secretion of the dairy cow and its application to the analysis of experimental data.

As indicated by the statement of the law, the percentage decline in milk flow from month to month should be fairly constant. However, some slight variations usually occur and therefore the best quantitative index of the persistency of production is the average per cent of persistency of secretion during the decline of milk flow.   The method of calculating the average persistency of the group of non-pregnant cows will be used as an illustration of this method.

The milk production for the month of maximum production was 1052 pounds while the following month was 998 pounds.   The percentage of the previous month's production may be obtained

TABLE 1

Persistency of milk secretion of non-pregnant cows

MONTH OF LACTATION	NUMBER ANIMALS	MONTHLY MILK PRODUCTION	PERCENTAGE OF PREVIOUS MONTH'S MILK PRODUCTION (PERSISTENCY INDEX)
2	920	1052	
3	912	998	94.86
4	923	938	93.98
5	914	879	93.71
6	917	833	94.76
7	912	792	95.07
8	904	752	94.94
9	905	715	95.07
10	906	676	94.54
11	852	650	96.15
12	653	617	94.92
Average...			.......94.80

by dividing each month's production by the production of the preceding month.   Thus,

$$\frac{998}{1052} \times 100 = 94.86 \text{ percentage of previous month's production}$$

The results are shown in table 1.

The average persistency index of this group of cows was 94.8 per cent.   The persistency of fat secretion during the lactation period can be determined in a similar way.   Due to the fact that the per cent of fat increases during the lactation as the milk

flow declines, the persistency of fat secretion is greater than is the persistency of milk secretion. In comparing individuals or groups of individuals it is important that comparisons be made only of either one or the other and not confuse persistency of milk secretion with persistency of fat secretion.

As working hypothesis it is assumed that each cow inherits a definite maximum persistency of secretion. Under favorable conditions she may closely approach her inheritance as regards this character but with poor feed and management, her potential persistency of secretion may not be fully expressed and the persistency index will be low.

One indication that there is a definite upper limit in the persistency of secretion is shown by the fact that overfeeding will not increase the persistency of secretion (4). The surplus nutrients consumed are stored on the body as indicated by an increase in live weight.

Having shown that the persistency of secretion is remarkably constant under controlled conditions, it may be used as a guide in evaluating the effect of unfavorable conditions on milk secretion. For example, in the case of the effect of pregnancy on the secretion of milk, it was noted that when cows are bred the third and fourth months of lactation, the last three months of lactation show a greater decline than the previous month's decline. This indicates the extent of the effect of pregnancy on milk secretion. At the same time it shows that these last months can not fairly be included in determining the average persistency index.

The frequency of milking is another factor which would affect the average persistency index of the dairy cow. Quantitative data on the effect of four, three, and two times a day milking on the persistency index will be of great value. However, unless the same cows are used in each classification, there will be danger of getting a more persistent group of animals in the classes with the greatest frequency of milking.

From the preceding discussion it will be seen that there are two factors in operation. To obtain a persistency index which will best represent the inheritance of the animal for persistency of milk secretion, it is necessary to exclude that part of the lactation

which is markedly influenced by environmental factors which may be noted by marked deviation from the average. On the other hand, the amount of deviation from the average is an excellent index of the effect of environmental factors of various kinds on milk secretion.

The two factors chiefly concerned in total milk and fat production are the yield during the maximum month (a month is used as a convenient unit) and the persistency of production or the rate of decline. At a given maximum month's production, the cause of variations in total yield of milk or fat are due to the variations in the rate of decline. This being true, it is possible to obtain an index of persistency by determining the ratio between the total yearly milk or fat production and the maximum month's production. For example, if the total yearly production were 600 pounds of fat and the fat production during the maximum month were 50 pounds, then the ratio $\frac{600}{50}$ would be 12. This would mean that each month's production was, on the average, 50 pounds of fat and that the cow was 100 per cent persistent. In other words, a ratio of 12 for a yearly record would be equivalent to 100 per cent of persistency. For a ten months or three-hundred-and-five-day record, a ratio of 10 would be equivalent to 100 per cent persistency.

In order to interpret, in terms of persistency, ratios obtained which are less than 12 or 10 as the case may be, table 2 was prepared. It shows the rate of decline of fat production with varying percentages of persistency. Taking 100 pounds of fat as the maximum for the second month, the fat secreted each month with varying rates of decline in persistency from 100 per cent down to 85 per cent are presented. A slight possible error, may be introduced by assuming that the first and third months would be similar. It was used because there are indications that a close relation exists between the rate of increase of milk secretion and the rate of decline of milk secretion during the lactation period.

The total yield of fat for the ten months and also for the year

TABLE 2

The decline of fat secretion with varying per cent of persistency

MONTH OF LACTATION	100	99	98	97	96	95	94	93	92	91	90	89	88	87	86	85
	lbs. fat	lbs. fat	lbs. fat	lbs. fat	lbs. fat	lbs. fat	lbs. fat	lbs. fat	lbs. fat	lbs. fat	lbs. fat	lbs. fat	lbs. fat	lbs. fat	lbs. fat	lbs. fat
1	100	99.00	98.00	97.00	96.00	95.00	94.00	93.00	92.00	91.00	90.00	89.00	88.00	87.00	86.00	85.00
2	100	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
3	100	99.00	98.00	97.00	96.00	95.00	94.00	93.00	92.00	91.00	90.00	89.00	88.00	87.00	86.00	85.00
4	100	98.01	96.04	94.09	92.16	90.25	88.36	86.49	84.64	82.81	81.00	79.21	77.44	75.69	73.96	72.25
5	100	97.03	94.12	91.27	88.47	85.74	83.06	80.44	77.87	75.36	72.90	70.74	68.16	65.85	63.60	61.41
6	100	96.06	92.24	88.53	84.94	81.45	78.07	74.80	71.64	68.58	65.61	62.74	59.97	57.29	54.70	52.50
7	100	95.10	90.39	85.87	81.54	77.38	73.39	69.48	65.91	62.40	59.05	55.84	52.77	49.84	47.04	44.37
8	100	94.15	88.59	83.30	78.28	73.51	68.99	64.61	60.63	56.79	53.15	49.70	46.44	43.36	40.45	37.71
9	100	93.21	86.81	80.80	75.14	69.84	64.85	60.09	55.78	51.68	47.83	44.23	40.87	37.73	34.79	32.05
10	100	91.35	85.08	78.34	72.14	66.34	60.96	55.89	51.32	47.03	43.05	39.37	35.96	32.82	29.92	27.24
Total..........	1,000	963.84	929.27	896.20	864.67	834.51	805.86	777.80	751.79	726.65	702.59	679.83	657.60	636.58	616.48	587.26
Ratio of total/maximum	10.00	9.63	9.29	8.96	8.64	8.34	8.05	7.77	7.51	7.26	7.02	6.79	6.57	6.36	6.16	5.87
11	100	90.44	83.38	76.02	69.25	63.03	57.30	51.97	47.21	42.79	38.74	35.04	31.65	28.56	25.73	23.16
12	100	89.54	81.71	73.74	66.48	59.87	53.86	48.34	43.44	39.94	34.87	31.18	27.85	24.84	22.13	19.68
Total yearly fat production.......	1,200	1,143.82	1,094.36	1,045.96	1,000.40	957.41	916.84	878.11	842.44	809.38	776.20	746.05	717.10	689.98	664.34	640.11
Ratio of total/maximum	12.00	11.43	10.94	10.45	10.00	9.57	9.16	8.78	8.42	8.09	7.76	7.46	7.17	6.89	6.64	6.40

PER CENT OF PERSISTENCY

are given with the ratio of the total fat production for the period
to the maximum month's production.   These results are plotted
in figure 1.   From the figure it is possible to determine the per-
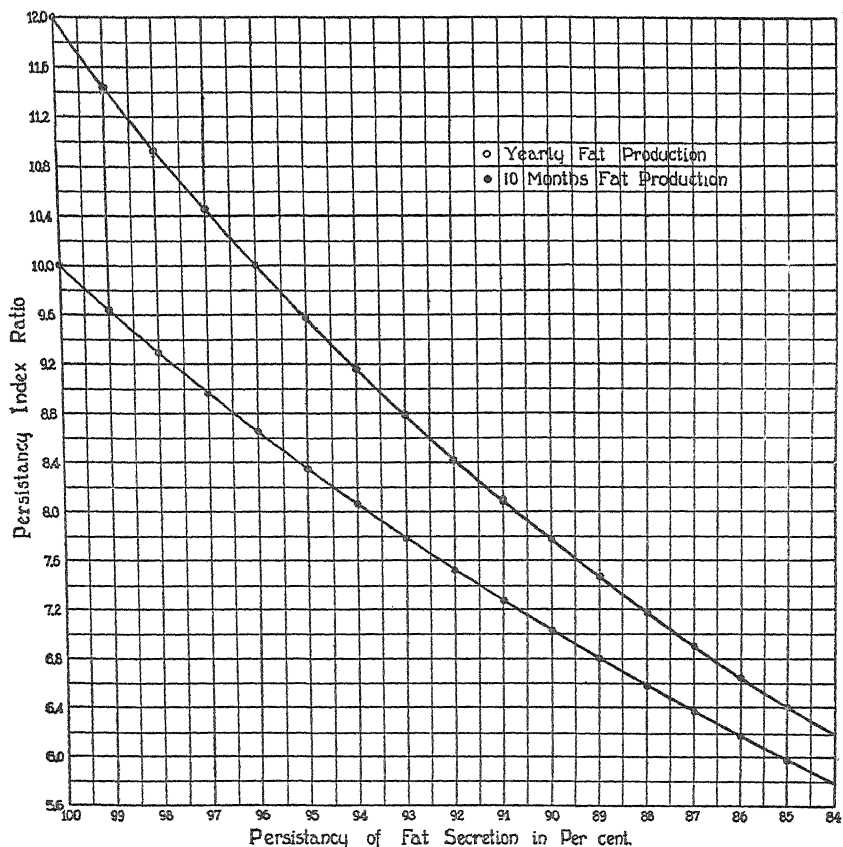
Fig. 1. Relation Between Persistency of Fat Secretion in Per Cent and the Persistency Index Ratio

It was plotted from the data in table 2. The persistency index ratio is the relation between the maximum month's fat production and the total fat production. The persistency of fat secretion in per cent is obtained by dividing each month's production by the production of the preceding month.

centage of persistency from any given value for the ratio either
of the ten months or years production.   For example, a ratio
of 9.6 for a yearly record is equivalent to 95.1 per cent of persist-
ency, a ratio of 10.8 is equivalent to 97.8 per cent.   For a ten-

months production, a ratio of 9.2 is equivalent to 97.7 per cent of persistency.

THE PREDICTION OF TOTAL PRODUCTION

The relation of the maximum month's production and persistency of secretion to total yearly production may be used in predicting total yearly production from the first part of the record. The accuracy of the method depends on the uniformity of the rate of decline of milk secretion. If the environmental conditions are favorable and the decline in production is fairly uniform, an

TABLE 3

	DAYS	POUNDS FAT	PERCENTAGE OF PREVIOUS MONTH'S PRODUCTION
January......................	17	54.75	
February.....................	28	106.40	
March........................	31	102.28	99.5
April........................	30	100.68	98.4
May..........................	31	102.46	101.7
June.........................	30	92.74	90.5
			(Ave. 4 months, 97.5)
July.........................	31	94.35	101.7
August.......................	31	85.36	90.4
			(Ave. 6 months, 97.0)
September....................	30	75.02	87.8
October......................	31	72.32	96.3
November.....................	30	70.98	98.1
December.....................	31	73.34	103.3
January......................	14	33.45	
		1,064.12	96.77 (ave.)

accurate prediction can be made. The method can be illustrated by the record of a 1000-pound cow selected at random (table 3).

Let us assume first that only the first four months (after the maximum) are known. To estimate the total production, it is first necessary to determine the average persistency index during this period (obtained by determining the percentage of the previous production month by month). In this case it is 97.5. The persistency index is converted to the corresponding ratio by reference to figure 1. The ratio of 97.5 is 10.6. The ratio is

then multiplied by the production during the maximum month resulting in the estimated total yearly production. Thus,

$$106.4 \times 10.6 = 1127.8 \text{ pounds of fat}$$

Referring to the actual production, 1064 pounds of fat, the estimated production is found to be 6 per cent too high. If the first six months are used, the estimated production would be 1106.6 pounds of fat or 4 per cent too high.

VARIATION IN PERSISTENCY OF DAIRY COWS

To obtain an idea of the variation in the persistency of dairy cows, table 4 is presented. The relation between the maximum month and total yearly production is shown. It will be noted that there is considerable range in yearly production with cows producing the same amount of fat during their maximum month. As an illustration, the total production of the cows averaging 52.5 pounds of fat during their maximum month may be cited. It will be seen that the extremes of production are 363 pounds and 663 pounds, a difference of 300 pounds of fat. It is hardly conceivable that persistency would cause such a large difference. The numbers of these cows is limited. However, 113 cows or 12.2 per cent of the 928 cows in the group produced less than 425 pounds of fat, 209 cows or 22.5 per cent produced less than 450 pounds, 334 cows or 35.9 per cent produced less than 475 pounds of fat and 470 cows or 50.6 per cent produced less than 500 pounds of fat. On the other hand, 56 cows or 6 per cent produced above 575 pounds of fat, 151 or 16.3 per cent produced above 550 pounds of fat, and 283 cows or 30.5 per cent produced above 525 pounds of fat. There is a variation of more tha 100 pounds of fat by 56 per cent of the animals having an average month's maximum fat production of 52.5 pounds. The importance of persistency of production as related to total yearly production is strikingly shown. It also illustrates the importance of having a quantitative method of expressing this important characteristic.

TABLE 4

The relation between the maximum month's and total production, Guernsey cattle

TOTAL YEARLY FAT PRODUCTION, POUNDS

FAT PRODUCTION (MAXIMUM MONTH) *pounds*	362.5	387.5	412.5	437.5	462.5	487.5	512.5	537.5	562.5	587.5	612.5	637.5	662.5	687.5	712.5	737.5	762.5	787.5	812.5	837.5	862.5	887.5	912.5	937.5	962.5	982.5	Total
32.5	13	1																									14
37.5	43	48	32	7	2																						132
42.5	46	99	108	102	64	17	1																				437
47.5	26	59	109	122	154	121	86	30	3	1																	711
52.5	10	40	63	96	125	136	175	132	95	39	13	2															928
57.5	6	14	37	63	63	91	107	145	126	113	81	43	2	1													899
62.5			12	24	24	48	79	79	82	93	100	97	9	45	14	2											783
67.5			0	11	10	16	25	34	45	47	70	77	84	92	67	31	11	3									603
72.5			2	2	5	8	12	13	17	28	38	40	64	46	49	45	30	11	6								387
77.5						1	7	7	10	9	10	17	32	14	17	19	29	25	21	3	5						237
82.5								0	6	1	6	4	22	3	7	14	8	16	13	20	14	4	1	1			131
87.5								1	1	1	3	6	20	1	3	7	7	8	6	10	3	7	1	1	1	1	73
92.5													3	1	1	3	4	0	5	6	7	4	3	7	1	2	43
97.5													3		2	1	1	0	1	3	0	2	6	4	2	1	9
102.5																	1		0	3	1	0	0	0	0		5
107.5																			4	1		0	0	1			4
Total	144	261	363	427	447	438	492	441	385	332	321	286	239	203	160	122	91	63	57	46	30	17	10	13	4	4	5,396

Correlation coefficient 0.761 ± 0.004.

USES FOR PERSISTENCY INDEX

In comparative feeding trials it is important to determine the persistency of production (as indicated by previous lactations) of each cow and so group the lots of cows as to include animals of similar persistency in each lot. It seems possible that by increasing the sizes of the lots of cows and selecting the lots of cows carefully on the basis of their persistency of secretion, that continuous feeding trials without reversal would be practical. The effect of long continued experiments in which the effect of the previous feed would be eliminated as a factor are highly desirable. This would bring feeding trials of dairy cattle to a more exact basis and would be similar to the present method of feeding experiments with small animals with growth and reproduction as an index of the nutritive completeness of the feeds in question.

Are certain rations more efficient in maintaining the persistency of production of milk and fat, and permitting normal reproduction? The answer to this question can only be obtained by long continued, non-reversed feeding trials with cows evenly grouped according to their persistency of production in addition to the usual factors considered. The influence of various systems of management on persistency of production may also be studied.

From the standpoint of improvement of the milk and fat producing capacity of dairy cattle, the persistency index gives a quantitative value for persistency which can be used as a guide in the selection of breeding stock. Heretofore, the qualitative expression has been that a certain cow was persistent or that another lacked persistency, but this method enables one to say in a quantitative form that a cow is 97 per cent persistent or 93 per cent persistent as the case may be. The tremendous effect of persistency on the total yield of milk and fat which was shown in this paper, clearly indicates the need of considering persistency in the selection of breeding stock.

SHORT-TIME TEST LACKS PERSISTENCY INDEX

There has been much discussion of the merits and demerits of the short time test during the past few years. It appears to the

writer that the greatest criticism of such a short test is that it does not furnish an index of the persistency of the cow. If these two characteristics are not inherited together (high maximum production and persistency of production) and there is much evidence that such is not the case,[1] then continued selection for high maximum production is sooner or later going to produce cows excelling in the production of milk for a short time but lacking in the ability to continue that production for a full lactation period. The effect of such a course would be to materially decrease long time production.

RELATION OF CONFORMATION TO PRODUCTION

The question of conformation or type and production is one that has been much discussed but is a problem that has received little serious attention. Recently there has been an attempt to determine the relation between various external measurements and production. It seems reasonable that there should be a fairly close relation between a combination of these measurements and maximum production (seven to thirty days) because size of body and udder are needed for large production. Persistency of production, which so vitally influences long-time production, has few if any external signs by which its presence or absence may be indicated. For this reason it would appear that conformation can not bear a closer relation to yearly production than to maximum production.

SUMMARY

1. When all other conditions are uniform, the monthly milk or fat production during the lactation period after the maximum is passed, is a constant percentage of the preceding month's production.

2. Pregnancy, seasonal changes of environmental temperature, nutrition, and management may cause slight changes in the rate of decline of milk and fat secreted.

[1] An examination of table 4 shows that persistency of production and maximum of production are not closely correlated. Both low and high maximum production are found with low persistency and the same is true of high persistency.

3. Two methods for the quantitative determination of persistency of milk secretion are explained with a discussion of their relative merits.

4. The use of a combination of these methods in predicting total yearly production from the first part of the record by the use of the maximum month's yield and the persistency of production shown during that period was illustrated.

5. The uses of a quantitative index for persistency of secretion of milk or fat by dairy cattle in relation to problems of breeding, feeding, and management were indicated.

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INCREASED PRODUCING ABILITY IN DAIRY COWS DUE TO TEST CONDITIONS*

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Men who have handled large herds of cattle where official testing is carried on are generally close observers, and the statement has been made that when cows or heifers are placed on test they usually show development and increased producing ability as a result of the feeding and handling they receive during the period of the test. It would appear to be difficult to calculate the amount of development which might be attributed to the changed conditions surrounding the cow while on test, but a recent study of Guernsey and Jersey records throws some light on this matter.

It was originally planned to study the effect of age on production of butterfat by using only records of the cows having two or more tests. The thought was that the first record measured the cow's ability at a certain age, and the second record showed her ability at a later age. The difference would appear to be due to the increase in age between the tests. That this was not true was revealed when no indication was found showing when increasing age failed to improve production. It did not seem logical that age alone could be the cause of this continued improvement in the producing ability of cows after they had reached maturity, and for this reason the data were studied in a different manner.

Assuming that there was another factor besides age to be considered, the re-entry records were laid aside, and the initial records alone were sorted according to the ages at which the records were made. This gave the average production for initial-record cows at various ages, and since none of these were re-entry records the resulting averages for various age groups

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indicated the change in production brought about by age alone. Using these figures as a basis of the relationship of records of different ages, it was possible to adjust records to a comparable basis.

TABLE 1

Comparison of average initial and retest records of Jersey cows with initial records made while two to two and one-half years old

NUMBER OF COWS	AVERAGE INITIAL BUTTERFAT RECORD MADE AT 2 TO 2½ YEARS OLD	AVERAGE RE-ENTRY RECORD		INCREASE DUE TO AGE	PORTION OF RE-ENTRY RECORD DUE TO DEVELOPMENT	
		Age when made	Butterfat			
	pounds	*years*	*pounds*	*pounds*	*pounds*	*per cent**
240	384	3 – 3½	475	37	55	11.5
218	395	3½– 4	494	59	40	8.1
168	367	4 – 4½	496	72	57	11.5
152	391	4½– 5	553	92	70	12.7
105	388	5 – 5½	535	94	53	9.9
91	375	5½– 6	570	103	92	16.1
64	381	6 – 6½	548	108	59	10.8
43	382	6½– 7	561	108	71	12.7
36	365	7 – 7½	563	103	95	16.9
35	366	7½– 8	567	104	97	17.1
13	364	8 – 8½	589	103	122	20.7
12	332	8½– 9	549	94	123	22.4
3	322	9 – 9½	441	91	28	6.4
6	363	9½–10	632	102	167	26.4
2	279	10 –10½	490	79	132	26.9
2	393	11 –11½	732	95	244	33.3
2	365	13 –13½	528	88	75	14.2
1	396	14½–15	567	57	114	20.1
1	396	15½–16	683	57	230	33.7

* Weighted average per cent of this group is 11.9.

The study of the cows with two or more records was then resumed, and all records grouped according to ages. Then the animals with yearling records were regrouped according to the ages at which they were retested. In a like manner those with initial records made as junior two-year-olds, senior

two-year-olds, junior three-year-olds, etc., were sorted. The average initial record of each subgroup of cows was compared with the average initial record of all cows of the same age as previously determined. The next step was to calculate a record for these same cows at the age at which they were retested. This was done by adjusting the average initial record for that

TABLE 2

Comparison of average initial and retest records of Guernsey cows with initial records made while two to two and one-half years old

NUMBER OF COWS	AVERAGE INITIAL BUTTERFAT RECORD MADE AT 2 TO 2½ YEARS OLD	AVERAGE RE-ENTRY RECORD		INCREASE DUE TO AGE	PORTION OF RE-ENTRY RECORD DUE TO DEVELOPMENT	
		Age when made	Butterfat			
	pounds	*years*	*pounds*	*pounds*	*pounds*	*per cent**
46	424	3 – 3½	501	31	46	9.2
106	449	3½– 4	546	54	43	7.9
50	427	4 – 4½	541	70	44	8.1
70	435	4½– 5	552	74	43	7.8
36	418	5 – 5½	596	91	87	14.6
34	416	5½– 6	574	91	67	11.7
34	430	6 –6½	600	93	77	12.8
22	381	6½– 7	574	83	110	19.2
18	406	7 –7½	587	88	93	15.8
11	443	7½– 8	620	97	80	12.9
6	401	8 – 8½	576	88	87	15.1
10	402	8½– 9	595	88	105	17.6
2	390	9 – 9½	637	85	162	25.4
2	333	9½–10	565	72	160	28.3
2	390	10 –10½	541	85	66	12.2

* Weighted average per cent of this group is 10.7.

age, as previously determined, up or down in proportion to the amount by which the average initial record of these cows was more or less than the average initial record for all cows of the same age. For example, let us assume that a certain group of cows which were tested first as junior two-year-olds received their retests as senior three-year-olds. If the average initial

record of this group was 102 per cent of the average junior two-year-old initial records of all cows, then we may assume that if age alone were acting to increase production, when they were retested as senior three-year-olds they would still be capable of of producing 102 per cent of the average production of all cows tested initially as senior three-year-olds. Then 102 per cent of the average initial record of senior three-year-old cows would be the calculated record of this group at the age they were retested. This calculated record was compared with the actual retest records, and when these were found to be greater the difference was attributed to the development which these cows had undergone because of the handling while on previous tests.

Tables 1 and 2 set forth a detailed analysis of the two sets of Jersey and Guernsey cows with initial records as junior two-year-olds and later re-entry records. Each subgroup shows average re-entry records greater than would be expected if age alone were acting to increase the producing ability of these animals.

The study of a total of 1215 pairs of Guernsey records as outlined above showed that the average development factor for that group of animals was 12.2 per cent of the re-entry record.

Similarly, when 3722 pairs of Jersey records were studied, it was found that 11 per cent of the re-entry records of this group of animals was due to development.

The numbers are sufficiently large to make conclusions significant. The Jersey records were apportioned, according to ages at which the records were made, into 209 groups; and of this number only 23 groups, containing 39 of the total of 3722 individuals, failed to show a positive result. The Guernseys fell into 148 groups, of which only 9 groups, containing a total of 14 individuals out of the 1215, did not give a positive development factor.

Detailed results with complete tables for all groups are shown in Department Bulletin No. 1352.

THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE PERCENTAGE OF FAT IN COW'S MILK[*]

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The great variation in the composition of cow's milk is a matter of common observation. Studies of the causes of this variation, with regard to the fat content of milk, are numerous and have been summarized by Turner (30), who states that there are two fundamental causes of the variations. The first is the hereditary variation due to the genetic makeup of the breed and individual. The second cause appears to be due to physiological changes in the animal, caused by such factors as management, environment, lactation, reproduction and age. To the second cause Woodward (33) and Clothier (11) would add feed as a factor, and Eckles (12) adds the factor of condition of cow at time of calving. The effect of environment, or more specifically, environmental temperature, is considered in this paper.

The relation between season of the year and the per cent of fat in milk is generally known. It was shown by Eckles (13) in 1909 from a study of 240 lactation periods of cows in the Missouri and Iowa Experiment Station herds. He found that, regardless of when the lactation began, the percentage of fat when plotted followed a curve for the year, being lowest during June and July and gradually rising to the highest point in December and January, and then declining again till midsummer.

White and Judkins (32) concur in this opinion, presenting data taken on 49 cows over a period of seven and one-half years. They conclude that the milk tests lower in fat content in the

summer months than it does in the winter months, and that this variation is due to seasonal change.

In further work done at the Missouri station, Ragsdale and Turner (28) present data on 3763 Guernsey, 299 Jersey, and 95 Holstein-Friesian, yearly records made in official testing work. Their conclusions are identical with those of Eckles. Hooper (18) alone takes exception to this belief. From a study of 88 official Jersey records, one-fourth of them beginning in each of the four seasons of the year, he concludes that "the fat percentage runs lower while the cow is fresh regardless of the time of the year she freshens," and that "there is no indication that the fat percentage runs lower in July."

In all the work mentioned thus far the season of the calendar year was the only division in the data. Eckles, however, suggested that the temperature, which of course changes with the season, might be a possible cause of the variation. Hills (17) as early as 1891 conducted two trials using the local creamery butterfat tests of the milk of 30 herds in Vermont during the months of May, June, and July, 1891, and July, August and September, 1892. He says that "The results of both series of tests point strongly to the probability that, when cows are put on pasture, the per cent of fat rises as the temperature falls, and falls as the temperature rises. In other words, the percent of fat in milk varies inversely with the temperature changes."

There is, of course, a direct relationship between the season of the year and the temperature. The knowledge that summer is a time for low tests and winter a time for high ones therefore suggests temperature as a possible cause for the variation. Clothier (11), considering the differences in feeding practices between summer and winter seasons in Arizona, finds it "impossible to believe that the seasonal variations in butterfat content of milk, observed in Arizona, are not due directly to changes in feed;" however, all other workers on this subject suggest temperature as a probable cause, and two papers have been presented to show this, where attempts were made to control other conditions.

An early piece of work is reported by Brooks (9). During December, January, February, and March, 1895, he conducted an

experiment in warming a stable for cows.   Records were kept on three cows in each of two wings of his dairy barn.   One wing was equipped for hot water heat and kept at a temperature of 55°F. The other wing was not artificially heated, but because of the good construction, was seldom excessively cold.   He states "The most certain effect brought out by our experiments is the lowering of the per cent of fat in the milk in the warm stable."

In 1921 Ragsdale and Brody (27) reported a preliminary observation on ten cows, during the months of March and April. Their plan was to test out by direct observation the relation between the fluctuation of the environmental temperature and the fluctuation of the percentage of fat.   March and April are months in which a maximum fluctuation in temperature occurs in this state.   All the controllable conditions, such as feed and exercise, were kept approximately uniform throughout the period, the object being to record data showing the relation of temperature to the percentage of fat in milk, uninfluenced as far as possible under usual conditions, by other factors.   From their results they conclude that there is a relation between temperature and the percentage of fat, showing roughly an increase of about 0.2 per cent in the fat for a decrease of 10°F. in the temperature, between the observed temperature limits.

The daily herd fat tests were recorded against the outdoor temperature.   However, during stormy days and cool nights, the cows were kept in the barn, and while the barn was not heated artificially, it was undoubtedly warmer than the outdoor temperature.   Therefore the reported temperatures were not entirely correct for the environmental temperature under which the cows were kept.

DATA ON THE UNIVERSITY OF MISSOURI DAIRY HERD UNDER CONDITIONS OF NORMAL HERD MANAGEMENT

Because of the results obtained in the preliminary study by Ragsdale and Brody (27), more quantitative data were sought, eliminating the error already mentioned in that work.   Accordingly, in January, 1924, the recording of data for the entire dairy

herd of the University of Missouri was begun. Composite fat tests for all milk produced were taken daily, and a recording thermometer was used in obtaining the environmental temperature. When the cows were in the barn, the thermometer was placed in the barn; and when the cows were out of doors, the thermometer was placed out of doors. In this way the error in temperature reading, pointed out in the report of Ragsdale and Brody, was eliminated. All conditions of feeding, milking, care, and management remained normal, and the herd was handled in the usual way. Daily data for a period of 258 days was recorded. When the average daily temperatures are grouped into 10° intervals, as in table 1, the relation between the per cent of fat and the temperature is clearly shown.

TABLE 1

The daily data grouped into 10-degree intervals of temperature

NUMBER OF DAYS	AVERAGE TEMPERATURE FOR GROUP	AVERAGE PER CENT OF FAT
7	86.5	3.171
50	79.2	3.250
69	69.6	3.389
48	60.5	3.481
38	49.7	3.505
35	40.1	3.463
9	31.0	3.465
2	24.5	3.600

The gradual successive rise of the fat percentage in the temperature groups of 86.5°, 79.2°, 69.6°, 60.5°, 49.7°, and 24.5° was expected, but the drop in the 40.1° and 31° groups is unexplainable.

The 31° group is of little importance because of the small number of tests included in this average; and therefore the low average test of 3.465 per cent, while distinctly out of line with the other tests, may be somewhat discounted. However, in the 40.1° group there is a sufficiently large number of tests included, the number being 35. An explanation for this low test has not been found.

These data on the entire herd over such a long period of time,

undoubtedly contain some unavoidable errors due to the effect of some cows drying up and additional ones freshening throughout the year, but on the whole the number of cows of each breed in milk remained proportional during the entire period.

DATA ON TWO COWS UNDER CONTROLLED TEMPERATURE CONDITIONS

In the second part of the experimental work herein reported, an attempt was made to determine to what extent temperature was responsible for the variation in the fat tests, and also how much variation per unit change in temperature results.

The plan was to subject two similar cows to a constant temperature, until the percent of fat in the milk seemed at a constant level, and then to repeat the operation several times at 10° intervals of temperature, all conditions of milking and feeding remaining normal with the exception that drinking water was warmed to the experimental temperature before being offered to the cows.

The experimental animals were two mature Jersey cows as nearly alike in all respects as it was possible to get them. They were both in good flesh, and were of approximately the same nervous disposition.

For the moderate and warmer temperatures the cows were placed in a light, well ventilated room in the dairy barn, away from exciting influences. The temperature was regulated by a man in constant attendance and made more uniform by an electric fan which kept the air in motion. For the coldest temperatures the cows were placed in a large refrigerator room of a local packing house. This room was 50 by 70 by 12 feet in dimensions, and was electrically lighted. The cows were kept in a large dry compartment, but because of lack of space only one could be kept in the room at a time. It was planned to run trials at 90°, 80°, 70°, 60°, 50°, 40,° and 30°F., and to skip about from a high temperature to a low temperature in arranging the order of the trials. In this way the effect of acclimatization to any given range of temperature was eliminated. Tables 2 to 8 give the detailed numerical data on each trial.

TABLE 3
Trial I

This trial was run in the steam heated room. The planned experimental temperature was 90°F.

DATE (1924)	AVERAGE TEMPERA- TURE	COW 108		COW 121	
		Pounds milk	Per cent fat	Pounds milk	Per cent fat
November 1..................	92.0	21.1	7.204	24.0	6.420
November 2..................	91.7	21.5	5.750	23.5	5.927
November 3..................	91.9	21.7	5.284	23.3	4.811
November 4..................	92.3	21.7	5.078	25.1	4.845
November 5..................	93.5	18.3	5.085	25.9	4.979
November 6..................	93.7	19.1	5.513	24.6	5.344
November 7..................	94.8	18.3	4.685	24.7	4.831
November 8..................	92.3	19.9	5.155	20.5	5.211

Number days run... 8
Average temperature..92.7
Average pounds milk per day per cow..................................20.8
Average per cent fat.. 5.388
Average per cent fat eliminating first day of trial....................... 5.178

TABLE 3
Trial II

This trial was run in the steam heated room. The planned experimental temperature was 70°F.

DATE (1924)	AVERAGE TEMPERA- TURE	COW 108		COW 121	
		Pounds milk	Per cent fat	Pounds milk	Per cent fat
November 9..................	73.0	19.0	4.876	19.5	5.284
November 10.................	72.0	20.2	5.748	18.2	5.865
November 11.................	73.6	19.1	5.201	19.9	5.396
November 12.................	73.3	21.3	5.362	21.7	5.002
November 13.................	71.9	19.9	4.541	22.7	4.661
November 14.................	71.3	22.5	5.428	22.9	4.432

Number days run... 6
Average temperature..72.5
Average pounds milk daily per cow....................................20.5
Average per cent fat.. 5.149
Average per cent fat eliminating first day of trial......................5.163

TABLE 4

Trial III

This trial was run in the refrigerator room, one cow at a time. The planned experimental temperature was 40°F.

DATE (1924)	AVERAGE TEMPERA- TURE	COW 108		COW 121	
		Pounds milk	Per cent fat	Pounds milk	Per cent fat
November 20..................	44.5	17.1	5.377		
November 21..................	44.0	19.5	5.552		
November 22..................	44.0	19.4	5.756		
November 23..................	44.0	20.9	5.862		
November 24..................	43.7	19.4	5.642		
November 25..................	39.5	19.9	6.022		
November 26..................	40.5			22.0	4.790
November 27..................	38.0			21.0	6.550
November 28..................	37.5			21.5	7.046
November 29..................	38.0			22.0	6.390
November 30..................	40.5			22.0	6.529
December 1...................	39.0			19.0	6.992
December 2...................	36.5			20.0	6.780

Number days run—average of 6½ days for each cow.

Average temperature for entire period...................................39.9

Average pounds milk daily per cow.....................................20.2

Average per cent fat... 6.099

Average per cent fat eliminating first day of trial........................ 6.283

TABLE 5

Trial IV

This trial was run in the steam heated room. The planned experimental temperature was 50°F.

DATE (1924)	AVERAGE TEMPERA- TURE	COW 108		COW 121	
		Pounds milk	Per cent fat	Pounds milk	Per cent fat
December 3...................	52.1	20.6	5.919	20.8	5.884
December 4...................	52.7	20.3	5.754	21.8	6.094
December 5...................	51.2	20.0	5.210	22.4	5.453
December 6...................	53.1	21.0	5.342	23.2	5.568
December 7...................	52.7	19.5	5.622	23.6	5.616

Number days run.. 5

Average temperature..52.3

Average pounds milk daily per cow.....................................21.3

Average per cent fat..5.646

Average per cent fat eliminating first day of trial 5.582

In each of these trials it will be noted that the fat test was greatly out of line on the first day. A summary was made first using every day of each trial and another was made eliminating

TABLE 6

Trial V

This trial was run in the steam heated room. The planned experimental temperature was 60°F.

DATE (1924)	AVERAGE TEMPERA- TURE	cow 108		cow 121	
		Pounds milk	Per cent fat	Pounds milk	Per cent fat
December 8.....................	59.6	20.5	5.525	22.6	5.835
December 9.....................	60.3	19.6	5.124	20.0	5.204
December 10....................	61.0	19.6	5.386	22.1	5.409
December 11....................	62.8	20.5	5.412	23.5	5.500

Number days run...4
Average temperature...60.9
Average pounds milk daily per cow.......................................21.0
Average per cent fat.. 5.424
Average per cent fat eliminating first day of trial..................... 5.339

TABLE 7

Trial VI

This trial was run in the steam heated room. The planned experimental temperature was 80°F.

DATE (1924)	AVERAGE TEMPERA- TURE	cow 108		cow 121	
		Pounds milk	Per cent fat	Pounds milk	Per cent fat
December 13....................	79.0	19.0	5.415	23.0	5.856
December 14....................	80.7	19.7	5.007	23.3	5.257
December 15....................	80.5	20.7	4.598	23.9	5.557
December 16....................	79.8	19.8	5.015	23.1	5.111

Number days run...4
Average temperature...80.0
Average pounds milk daily per cow.......................................21.6
Average per cent fat.. 5.227
Average per cent fat eliminating first day of trial..................... 5.107

the first day of each trial. The comparisons were very similar in each case, however, and for this reason a summary is presented in table 9 using every day of every trial.

Throughout the running of these trials, rectal temperatures were taken and the humidity of the compartments was measured

TABLE 8

Trial VII

This trial was run in the steam heated room in very cold weather. The planned experimental temperature was 30°F.

DATE (1924)	AVERAGE TEMPERA-TURE	COW 108		COW 121	
		Pounds milk	Per cent fat	Pounds milk	Per cent fat
December 21...................	20.9	15.0	8.413	20.2	7.341
December 22...................	27.0	15.3	5.444	20.5	5.597
December 23...................	36.4	17.7	5.446	20.2	5.853
December 24...................	29.6	18.7	5.139	19.5	5.400
December 25...................	21.4	16.8	5.594	20.2	5.900

Number days run...5
Average temperature...27.0
Average pounds milk daily per cow.....................................18.4
Average per cent fat.. 6.012
Average per cent fat eliminating first day of trial........................ 5.543

TABLE 9

A summary of all of the controlled temperature trials

The trials are grouped successively by temperature changes in this table to show the comparison between fat tests.

TRIAL	TOTAL NUMBER OF DAYS	AVERAGE TEMPERATURE FOR ENTIRE TRIAL	AVERAGE POUNDS MILK PER DAY	AVERAGE PER CENT FAT FOR ENTIRE TRIAL
I	8	92.7	20.8	5.388
VI	4	80.0	21.6	5.227
II	6	72.5	20.5	5.149
V	4	60.9	21.0	5.424
IV	5	52.3	21.3	5.646
III	6½	39.9	20.2	6.099
VII	5	27.0	18.4	6.012

using a wet bulb thermometer. Body temperature of course remained normal and the relative humidity was about what would be found under normal average conditions.

DISCUSSION OF THE RESULTS
Regulation of body temperature

In seeking an explanation for the rather spectacular rise in the per cent of fat in milk, attendant upon decreasing environmental temperature, the relation between external temperature and body temperature is considered. Turner (30), in summarizing the factors which influence the per cent of fat in milk, states that variations may be brought about by physiological changes in the animal caused by environment. Lusk (22), in discussing the regulation of body temperatuie, points out that in cold-blooded animals body temperature varies directly with environment. The metabolism is very low at temperatures around 4°C., but increases rapidly with rising temperature. In warm blooded animals the body temperature is maintained at a constant level, independent of climatic conditions. This constant is one which is most favorable to the activity of nerve and muscle, and it is maintained in two ways:

1. Physical regulation. As the temperature rises the blood vessels of the skin become dilated, and heat is radiated from the skin. Evaporation of water from the body is also promoted. If the environmental temperature becomes so high that the physical regulation will not cool the body sufficiently, a supernormal temperature ensues and increased metabolism results.

2. Chemical regulation. In a cooling environment the contraction of the peripheral blood vessels, and the physical regulation will conserve the animal heat to a certain point. This point has been found to be 30°C. (86°F.) in guinea pigs. At this point the minimum of metabolism takes place and it is called the critical temperature. Below this point the metabolism is increased, producing more heat.

According to Barbour (1), equalization of temperature in the body is best attained through a mobile constituent such as water, which can circulate rapidly and freely. He mentions "water shifting," from blood to the brain, and vice-versa, as one of the factors in physical regulation. Osborne (26), in presenting some new aspects of the skin in temperature regulation, states that the

skin may be regarded as a gel containing water of imbibition. Evaporation of this water can occur independently of the sweat gland, and when the air is cold, dry, and in rapid motion, the evaporation may be contrary to the metabolic requirements of the body.

The effect of temperature on metabolism

In the chemical regulation of the body temperature, where more of the body tissue must be oxidized to furnish the needed heat, the metabolism of the individual is of course affected. Benedict (4) says that the basal metabolism of an individual is a function of the total mass of active protoplasmic tissue, and the stimulus to cell activity, at the time the measurement of the metabolism is taken. He mentions, as affecting basal metabolism, body weight, surface area, body composition, stimulus to cellular activity, sleep, and diurnal variation. Environmental temperature may be regarded as a stimulus to cellular activity, as was shown by Lusk (22), and it has a direct bearing upon the metabolism. Lusk quotes Rubner, who showed that extremes of temperature, both high and low, caused an increase in the basal metabolism, and that in the guinea pig 30°C. (86°F.) was the point where minimum metabolism took place.

Boothby and Sandiford (8), in a review of basal metabolism literature, state, "Experiments of Dubois show that an elevation of the temperature produces a rise in the metabolic rate of approximately 10 per cent for each degree Centigrade above the normal body temperature." Campbell and his associates (10) state that metabolism is raised by cool out-of-doors conditions, and that shivering is not necessary to raise the metabolism. McConnell and his associates (25) carried out a series of experiments with human beings to determine the correlation between metabolism and external temperature conditions. They say that, contrary to what might be expected, metabolism increases with exposure to high temperatures, Enough work has been done to substantiate this statement, but the relation remains yet to be found. Probably the difficulty in establishing this relation lies in the fact that in high temperatures the relative humidity and air movement

must be considered. They have used an "effective temperature" scale in studying the effect of heat on body metabolism. It is an index of the intensity of the heat felt by the body as a result of temperature, humidity, and air movement. Their conclusions are:

1. CO_2 produced and oxygen consumed increase with exposure to high and low temperatures.

2. Heat production increases with exposure to high and low temperatures.

3. There is a zone of minimum metabolism, between 75° and 83° effective temperatures, within which basal metabolism should be measured.

4. The metabolic rate becomes excessive when the temperature of the environment exceeds the body temperature.

The only work reviewed on the effect of temperature on the metabolism of ruminants was by Magee (24). A goat was subjected to various temperatures, from 30° to 100°F. and the metabolism rate calculated by indirect calorimetry. His conclusions were that

The critical range of temperature for the goat in this work was from 55° to 70°F. As the temperature falls below 55°F. metabolism exhibits a slight, gradually increasing, rise, due to the oxidation necessary to cope with the increasing heat loss. As the temperature rises above 70, metabolism shows a pronounced, gradual increase, owing to the gradually increasing efforts of the animal to promote heat dissipation by panting.

The relation between the fat content of the blood and the per cent of fat in milk

A theory that the per cent of fat in the milk may have a direct relation to the fat content of the blood naturally suggests itself. It has long been known that the fat content of the blood may vary greatly at times. Bloor (7), in working with dogs, found that, with dogs in good flesh, fasting from five to seven days produced a marked increase in the fat content of the blood. He states that after the first two or three days the fasting organism depends for

its support mainly on its fat stores. The increased use of fat would tend to increase the blood fat because of the greater amount transported. He quotes Schulz as saying that this increase may be as much as 50 to 100 per cent over the blood fat in animals that are being fed regularly. This increase, however, depends upon the nutritional condition of the animals; for, while animals in good flesh show the increase, when starved, thin animals fail to show it.

Another result of starving is an increased percentage of fat in the milk produced. Eckles (12) has shown that when the ration of a cow is insufficient to support her production, she loses weight rapidly, evidently drawing upon her body fat, and the test of the milk rises. This increase in test, he states, is due to the "transfer" of fat from the body to the milk, and is most marked immediately after calving.

Starvation, then increases the fat content of the blood and also of the milk. Gage and Fish (15) supply a link in an explanation of the rise in fat content of milk with lowering temperature. They fed mammals on fat that had been stained with a fat soluble dye (sudan III) and then traced its course through the life processes of the animals. The colored fat was found in the blood, and later in the milk. In the case of the cat it required but five hours to digest, absorb, and deposit the pink fat and to turn a part of it into milk fat. With the cow, however, no pink fat could be produced in the milk, but this work at least shows that the lipoids of the blood are transported and transferred directly into the milk.

It therefore seems reasonable to assume that anything which would bring about an increase of the fat or lipoid content of the blood would effect a resultant increase in the fat content of the milk. It has been shown by Barbour (1), and Lusk (22), and others that the hemoglobin, the red blood cell count and the viscosity of the blood increase under the influence of cold environment. Under the present conceptions, such changes are attributed to the fluid concentration of the blood, and if the blood becomes more "concentrated" the percentage of each solid is

increased accordingly and the fat in the blood is therefore increased.

Low temperatures, then, cause an increase of the fat content of blood and this may result in an increased per cent of fat in the milk.

SUMMARY AND CONCLUSIONS

1. Daily data on the University of Missouri dairy herd for a period of 258 days are presented showing the average fat test and environmental temperature. The temperatures ranged from 85.5° to 24.5°F., or a net range of 62°. The average fat tests ranged from 3.171 to 3.600 per cent, or a total difference of 0.489 per cent, and it was generally found that the lower the temperature the higher the fat test. This increase in the fat test amounted to 0.079 per cent for each 10° lowering of the temperature. However, because of the many variables, this result is not considered of as much significance as the results of the controlled temperature trials.

2. Seven controlled temperature trials, on two Jersey cows, were run at 10° intervals of temperature, with all other conditions remaining normal. The range of temperature was from 92.7° to 27°F. When the seven periods were taken as a unit, there was a total temperature range of 65.7°, and a total increase in the fat test of 0.624 per cent, or an average increase of 0.095 per cent for each 10° lowering of the temperature.

3. In the controlled temperature trials there was a constant increase in the per cent of fat as the temperature dropped below 70°F. From 72.5° to 27° there was a temperature range of 45.5° and a total increase of 0.863 per cent fat, or an average increase of 0.189 per cent fat for each 10° lowering of the temperature. This is almost exactly in accord with preliminary studies at this Station by Ragsdale and Brody, who reported a rise of 0.2 per cent fat for each decrease of 10°F., between the temperature units of 70° and 30°F.

4. Above 70°F., there was an actual increase in the fat test. The reason for this is not known, but it is believed to have been due to increased metabolism, induced by higher temperature

or the result of disturbing the animals by the sudden changes from one temperature to another.   The latter is indicated by the fact that the first day's test in each trial was abnormally high.   When these first days were eliminated the increase in the fat test was not nearly so pronounced.   It would seem that there is a range of temperature between 70° and 90°F. within which the lowest testing milk is produced.   A variation in the environmental temperature either way will bring about an increase in the per cent of fat in cows milk.

5. It is believed that the controlled temperature trials are more nearly indicative of the effect of environmental temperature on the percentage of fat in cows milk than the daily herd averages where there were many variables.   It is therefore concluded that all other conditions remaining constant, there is an increase of approximately 0.2 per cent fat in cows milk for each 10° lowering of the temperature within the limits of 70° and 30°F.   In the second part of this experiment all conditions, other than temperature were apparently effectively controlled and remained constant throughout every change in temperature.   Temperature, then, was the only variable.

6. It is concluded that temperature is a major factor in the seasonal variation of the per cent of fat in cows milk.

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SOME FACTORS AFFECTING THE GROWTH OF CERTAIN STRAINS OF P. ROQUEFORTI BLUE MOLD. II*

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The manufacturing process of Roquefort cheese and to a lesser extent that of Wensleydale cheese includes the practice of salting the curd or cheese (4) (5). The actual percentage of salt incorporated in the ripened cheese is shown in table 1.

Thom (6) shows the retarding effect of salt on the growth of thirty-one species of mold. He further demonstrates that P. roqueforti has a high salt tolerance, but the strain used was isolated from Roquefort cheese where a high percentage of salt is incorporated (6). Therefore, it is conceivably possible that wide variations in salt tolerance might exist with the different strains of P. roqueforti as isolated from Wensleydale and Roquefort cheese.

In an investigation into the manufacture of Wensleydale cheese (4) it was noted that the cheese inoculated with culture 1 (3) grew quicker and more abundantly than that inoculated with culture 16 (3). In each case the cheese contained the same percentage of salt—approximately 1 per cent (4).

OBJECT

The object of this work is to measure the rate of growth of several cultures of different strains of P. roqueforti as influenced by salt.

CULTURES USED

The cultures of P. roqueforti used were:

No. 1. P. roqueforti secured from the Dairy Division, Washington (3).
No. 16. Isolated from Wensleydale (3).
No. 32. Isolated from Wensleydale cheese inoculated with culture 16 (4).
No. 33. Isolated from Wensleydale cheese inoculated with culture I (4).

TABLE 1

AUTHORITY	REFERENCE	ORIGIN OF CHEESE	NUMBER OF ANALYSES	PER CENT MOISTURE	PER CENT ASH	PER CENT SALT
Wensleydale cheese						
Chattaway..........	1		1	28.30	3.7	
Golding............	4	Imported cheese made by Rowntree, Yorks, England	4	28.76	1.74	0.12
Golding...........	4	Made at University of British Columbia	8	24.82	3.21	0.99
Roquefort cheese						
Matheson..........	5	Roquefort from France	?	38.0	6.0	4.0
Golding............	4	Roquefort from cow's milk. Made at University of British Columbia	?	36.0	4.77	3.33

MEDIA

Milk. Sweet skim milk in which no bacterial change had taken place was used in every case. Test tubes of uniform diameter were selected, filled with exactly 10 cc. of milk, plugged and sterilized.

Milk + salt. A good grade dairy salt of 99 per cent purity was used. The dried salt was weighed into sufficient dry test tubes in amounts of 0.4, 0.8, 1.2, and 1.6 grams per tube. These tubes were plugged and dry sterilized. When both the 10 cc. tubes of milk and salt were cool, the salt was added to the milk
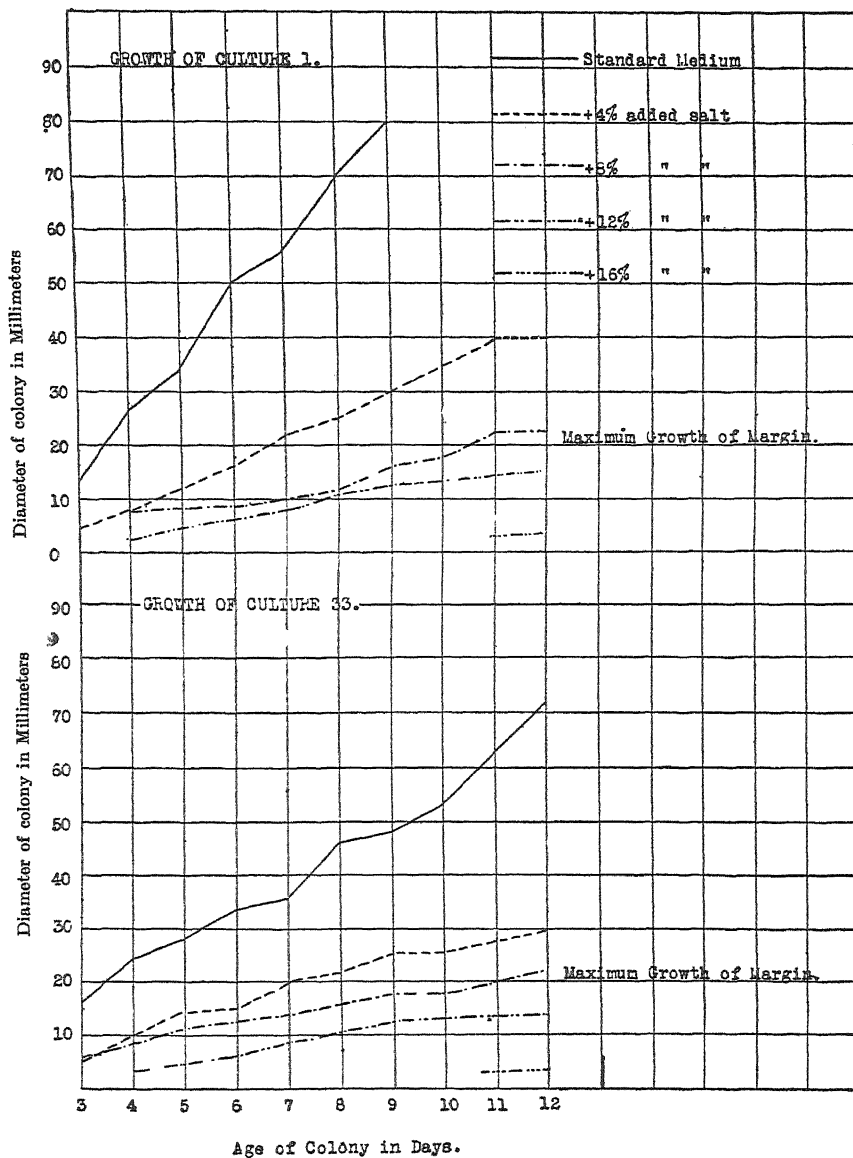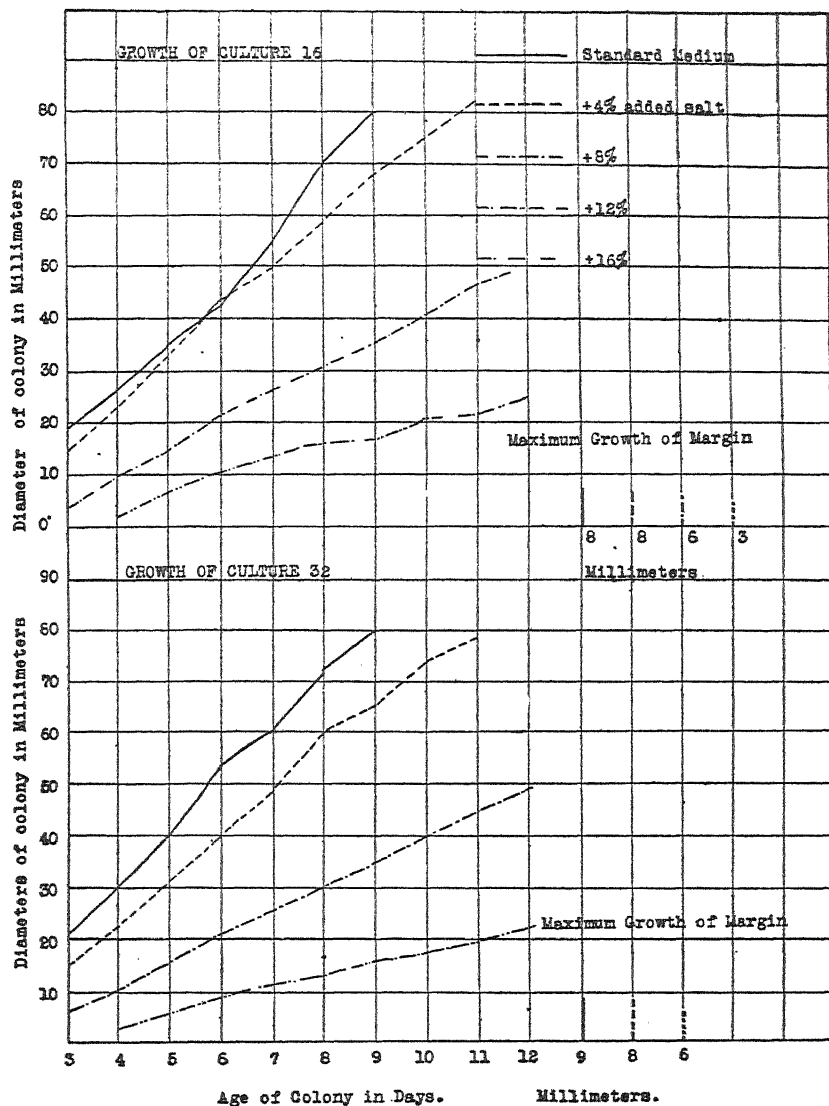
FIG. 1

FIG. 2

under sterile conditions. Thus concentrations of salt and milk were obtained of the following strength 4, 8, 12 and 16 per cent added salt.

Standard medium. The standard medium used in this investigation was the same as that previously employed (2).

Standard medium + salt. To the standard medium dairy salt was added in concentrations of 4, 8, 12 and 16 per cent added salt. The media were then plugged and sterilized in the usual way.

METHODS

The methods employed on inoculation, incubation, measurement of growth in milk, and measurement of growth on solid media are specifically defined in "Some factors affecting the growth of certain strains of P. roqueforti, blue mold, I" (2).

THE SALT TOLERANCE OF STRAINS OF P. ROQUEFORTI IN MILK

Five media composed of milk and milk with different concentrations of salt as given were used in this experiment (table 2).

Quantitative determinations were made of the degree to which the cultures 1 and 16, respectively, digested the casein of these milks.

The conclusions to be drawn from table 2 are:

TABLE 2

Different strains of P. roqueforti grown in milk and milk + varying per cent of salt

Cultures grown at room temperature in test tubes for ten days

CULTURES	MILK		MILK + 4 PER CENT ADDED SALT		MILK + 8 PER CENT ADDED SALT		MILK + 12 PER CENT ADDED SALT		MILK + 16 PER CENT ADDED SALT	
	Per cent casein	Per cent of casein digested*	Per cent casein	Per cent of casein digested*	Per cent casein	Per cent of casein digested*	Per cent casein	Per cent of casein digested*	Per cent casein	Per cent of casein digested*
Uninoculated control	3.10									
Culture 1	2.20	29.0	2.27	26.8	2.50	19.4	2.76	11.0	2.76	11.0
Culture 16	2.79	10.0	2.91	6.1	2.97	4.2	2.97	4.2	2.95	4.8

* This figure represents the percentage of the total casein that has been rendered soluble.

1. Culture 1 (Roquefort origin) has a greater power to digest casein in milk and salted milk than has culture 16. This is in part confirmed by previous work (3).

2. The power of culture 1 to grow and digest casein in the higher concentrations of salt is relatively less than is the case with culture 16.

3. Concentrations of salt as found in Roquefort cheese would retard but not inhibit the growth of either strain of P. roqueforti.

THE SALT TOLERANCE OF STRAINS OF P. ROQUEFORTI IN THE STANDARD MEDIA

Five media composed of the standard medium and the standard medium with different concentrations of salt were used in this experiment (figs. 1 and 2). The rate of growth has been expressed graphically for the four cultures (see figs. 1 and 2).

The conclusions to be drawn from figures 1 and 2 are:

1. Salt retards the growth of all four cultures of mold.

2. Cultures 1 and 33 of Roquefort origin are more retarded in growth by high concentrations of salt than are cultures 16 and 32 of Wensleydale origin.

From the other data recorded, the following facts are of significance:

1. Cultures 1 and 33 showed a stunted growth with abnormal spore formation in as low a concentration as 4 per cent added salt.

2. Cultures 16 and 32, though showing a stunted growth, still retained their more or less typical appearance with green spores in the medium containing 12 per cent added salt.

SUMMARY

The result of the work recorded in this paper, showing the retarding effect of salt on the media defined, would suggest that salt concentrations, as usually found in Roquefort cheese, may retard but not inhibit the growth of the mold of either strain.

With Wensleydale cheese, where the percentage of salt in the cheese is lower, the salt would not be expected to be an important inhibiting factor.

The failure of culture 16 to grow as well as culture 1 in Wensleydale cheese must be accounted for by other factors than salt.

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AN ALGEBRAIC METHOD OF PROPORTIONING ICE CREAM MIXES*

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This discussion explains a method of proportioning and standardizing ice cream mixes which has been presented to students in the Department of Dairy Industry at Cornell University each year since 1921. The flexibility of its application to any mix requirements and the simplicity of the calculations have received the approval of all classes of students interested in ice cream making. Continued use of the method under commercial conditions where speed and accuracy are essential has demonstrated its usefulness.

Materials which are used in making ice cream may be divided into two classes; milk products and those products which are not derived from milk. The milk products furnish, generally speaking, the milk fat, milk solids not fat (msnf), and the bulk of the water in the mix. Trade demands and marketing conditions require the presence of definite amounts of sugar, gelatin, flavors, etc., so that it is by correctly proportioning the amounts of milk products that the desired amount of fat and milk solids not fat are obtained in the ice cream.

To simplify the explanation of this method of standardizing, the solution of a typical problem will be demonstrated.

Under conditions which are assumed, it is necessary to make 1000 pounds of ice cream mix containing 10 per cent fat, 11 per cent msnf, 13.5 per cent sugar, and 0.5 per cent gelatin dissolved in 6 per cent water.

The available materials and their composition are: Any amount of:

	FAT	MSNF	SUGAR	GELATIN
	per cent	*per cent*	*per cent*	*per cent*
Cream..................................	40	5.4	.	
Whole milk.............................	4	8.8		
Skim condensed........................	0.1	27.0		
Sugar..................................			100	
Gelatin................................				100

The first step of the solution consists in determining the amounts of the materials not milk which must be used in the mix.

$$
\begin{aligned}
&1000 \text{ pounds mix} \times 0.135 = 135 \text{ pounds sugar} \\
&1000 \text{ pounds mix} \times 0.005 = 5 \text{ pounds gelatin} \\
&1000 \text{ pounds mix} \times 0.06 = 60 \text{ pounds water} \\
&\text{Total weight of non-} \\
& \text{milk products} = 200 \text{ pounds}
\end{aligned}
$$

gelatin solution

The difference between the total weight of the mix desired, 1000 pounds, and the weight of the ingredients other than milk in the mix, 200 pounds, indicates that the cream, whole milk, and condensed milk must furnish a total of 800 pounds.

It is evident that the difference between the total weight of the milk products in the mix and the combined weights of cream and condensed milk, will equal the weight of the whole milk. Stated as an equation this fact appears as:

800 — pounds of cream — pounds of condensed milk = pounds of whole milk

If pounds of cream = x and pounds of condensed milk = y, then

$$800 - x - y = \text{pounds of milk}$$

Using the above symbols again, it is possible to state that:

The pounds of fat derived from the cream = 40 per cent of x.
The pounds of fat derived from the condensed = 0.1 per cent of y.
The pounds of fat derived from the milk = 4 per cent of $(800 - x - y)$.

The desired mix must contain 10 per cent fat, or 1000 times

0.10, which is 100 pounds of fat.   Since the above milk products furnish all the fat in the mix, the Fat Equation can be formed.

$$\text{Fat equation } 0.40x + 0.001y + 0.04 (800 - x - y) = 100$$

It is possible by the same reasoning to state that:

The pounds of milk solids not fat derived from the cream $= 5.4$ per cent of x
The pounds of milk solids not fat derived from the condensed $= 27$ per cent of y
The pounds of milk solids not fat derived from the milk $= 8.8$ per cent of $(800 - x - y)$.

The desired mix must contain 11 per cent milk solids not fat, or:

$$1000 \text{ times } 0.11 = 110 \text{ pounds of Msnf}$$

Since the milk products furnish all the msnf, these facts may be used to form the msnf equation.

$$\text{Msnf equation } 0.054x + 0.27y + 0.088 (800 - x - y) = 110$$

The solution of these equations may be arrived at through methods known to any one acquainted with elementary algebra. A simple procedure is indicated here.

The cumbersome decimals of these equations may profitably be eliminated by multiplying the numbers of each equation by 100.

$$\text{Fat equation } 40x + 0.1y + 4 (800 - x - y) = 10{,}000$$
$$\text{Msnf equation } 5.4x + 27y + 8.8 (800 - x - y) = 11{,}000$$

Simplifying and combining:

$$\text{Fat equation } 36x - 3.9y = 6800$$
$$\text{Msnf equation } -3.4x + 18.2y = 3960$$

One of the unknown quantities must be eliminated.   To accomplish this multiply each number of the fat equation by the first number of the msnf equation,

$$36x - 3.9y = 6800$$
$$3.4$$

$$\text{Fat equation } 122.4x - 13.26y = 23{,}120$$

Then multiply each number of the msnf equation by the first number of the fat equation.

$$-3.4x + 18.2y = 3960$$
$$36$$

Msnf equation $-122.4x + 655.2y = 142,560$

Adding the fat and msnf equations, the values for x cancel each other,

Fat equation $122.4x - 13.26y = 23,120$
Msnf equation $-122.4x + 655.2y = 142,560$

$$641.94y = 165,680$$
$$y = 258.092 \text{ pounds of condensed milk}$$

Substituting this value for y in

Fat equation $36x - 3.9y = 6800$, then
Fat equation $36x - 3.9 \times 258.092 = 6800$

Combining and transposing,

$$36x = 7806.55$$
$$x = 216.848 \text{ pounds of cream}$$

Since

$$800 - x - y = \text{pounds of milk}$$

therefore

$$800 - 216.848 - 258.092 = 325.06 \text{ pounds of milk}$$

Formula for desired mix

	MATE-RIALS	FAT	MSNF	SUGAR	GELA-TIN	WATER
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
Cream...........................	216.848	86.7392	11.7097			
Whole milk......................	325.06	13.0024	28.6052			
Condensed milk..................	258.092	0.2580	69.6848			
Sugar...........................	135			135		
Gelatin.........................	5				5	
Water...........................	60					60
Total...........................	10000	99.9996	109.9997			
Desired.........................	10000	100	110	135	5	60

It may be necessary to incorporate certain odd lots of milk, cream, or condensed milk into a mix in addition to the regular ingredients. To accomplish this, determine the pounds of fat, milk solids not fat and sugar furnished by each small lot of product to be used and deduct these amounts from the amount of fat, milk solids not fat, and sugar required by the mix. Deduct the total weight of each lot of milk, cream, or condensed from the calculated weight of milk products required by the mix. The mix can then be proportioned by using the proper amounts of the milk products which must furnish the remaining amounts of fat and milk solids not fat of the mix. The amounts of these milk products can be calculated by the use of the equations whose derivation has already been explained.

Sometimes sweetened condensed milk must be used in making ice cream. Under such conditions the condensed will furnish part of the sweetening of the mix. Two methods of calculation may be used under such circumstances. In the first place the mix may be calculated in exactly the manner explained in the original problem by disregarding temporarily the sugar content of the condensed. When the amount of each milk product has been determined then the pounds of sugar in the condensed should be deducted from the calculated amount of cane sugar necessary. The difference equals the pounds of sugar which must be added to the mix. As an example, it may be assumed that a certain set of conditions requires the addition of 300 pounds of condensed milk containing 120 pounds of sugar to a mix which needs 400 pounds of sugar for sweetening. In this case it is necessary to add 280 pounds of sugar. This obviously leaves the mix 120 pounds short of the correct weight, and this difference in weight must be corrected by the addition of water.

When it is undesirable to add water to a mix and sweetened condensed must be used, the procedure must be varied slightly. Assume, for example, that 100 pounds of sweetened condensed contains 40 per cent sugar, 6 per cent fat, and 30 per cent msnf. If the sugar could be removed, there would remain 60 pounds of a theoretical condensed milk containing 10 per cent fat and

50 per cent msnf. The percentage composition of the theoretical condensed milk can then be used in calculating the necessary amounts of milk and cream by means of the equations which have been explained. The amount of theoretical condensed milk represents only 60 per cent of the real condensed milk. Therefore, the weight of this theoretical condensed milk calculated as necessary for the mix must be multiplied by 100/60 to obtain the true amount of condensed milk to be used in the mix. The difference between the theoretical and the real weight of the condensed milk equals the weight of the sugar which must be deducted from the total amount required to sweeten the mix.

When the mix ingredients are placed in a mixing tank, it is common practice to determine the composition of the whole mix. It may happen that it does not conform to the desired percentage composition. This inaccuracy may be due to one or both of two errors; inaccurate analysis of the ingredients which were used in calculating the formula, or inaccurate weighing of the ingredients at the time of mixing. When corrections are necessary, the same procedure may be used which was explained in connection with the use of sweetened condensed milk. The mix must be combined with the products which will correct its inaccuracy. In forming the equations, it is best to use the percentage composition of a theoretically non-milk-product free mix. For example a mix which is composed of 10 per cent fat, 11 per cent msnf, 14.5 per cent sugar, and 0.5 per cent gelatin contains 15 per cent or 15 pounds per hundred of non-milk products. The weight of theoretical non-milk-product free mix, therefore, equals 85 pounds. The percentage composition of this theoretical non-milk-product free mix may be expressed as $\frac{10}{85} \times 100$ or 11.7 per cent fat and $\frac{11}{85} \times 100$ or 12.9 per cent msnf. The remaining calculations are identical with the procedure of proportioning a mix in which a theoretically sugar free condensed milk is used as one of the ingredients.

Some workers prefer to use formulas in the proportioning of a mix. Under such circumstances the following expressions for the amounts of necessary materials may be found useful. These

expressions are solutions of the equations already described in which letters are used in place of numbers.

x = pounds of cream or butter, etc.
y = pounds of condensed or powdered milk, etc.
m = pounds of milk products in the mix
$m - x - y$ = pounds of milk or skimmilk, etc.
a = per cent of fat in cream, etc.
b = per cent of fat in condensed milk, etc.
c = per cent of fat in milk, etc.
d = per cent of msnf in cream, etc.
e = per cent of msnf in condensed milk, etc.
f = per cent of msnf in milk, etc.
F = pounds of fat desired in the mix
S = pounds of msnf desired in the mix

$$x = \frac{(F - cm)\ (e - f) - (S - fm)\ (b - c)}{(a - c)\ (e - f) - (d - f)\ (b - c)} = \text{pounds of cream, etc.}$$

$$y = \frac{(a - c)\ (S - fm) - (d - f)\ (F - cm)}{(a - c)\ (e - f) - (d - f)\ (b - c)} = \text{pounds of condensed milk, etc.}$$

$$m - x - y = \text{pounds of milk, etc.}$$

The application of these formulas may be illustrated by the problem which has been solved in this discussion by substituting in each formula the proper values of the letters.

Substituting in the expression for the pounds of cream:

$$x = \frac{[100 - (0.04 \times 800)]\ [0.27 - 0.088] - [110 - (0.88 \times 800)]\ [0.001 - 0.04]}{(0.40 - 0.04)\ (0.27 - 0.088) - (0.054 - 0.088)\ (0.001 - 0.04)}$$

Simplifying:

$$x = \frac{[(100 - 32) \times 0.182] - [(110 - 70.4) \times (- 0.039)]}{(0.36 \times 0.182) - (- 0.034 \times - 0.039)}$$

Simplifying:

$$x = \frac{12.376 + 1.5444}{0.06552 - 0.001326} = \frac{13.9204}{0.06419} = 216.84 \text{ pounds of cream}$$

It may be noted that the denominator in the formula for the value of y is identical with the denominator in the expression

for the value of x. The equation for the value of y can then be written as:

$$y = \frac{(a - c) \ (S - fm) - (d - f) \ (F - cm)}{0.06419}$$

Substituting:

$$y = \frac{[0.40 - 0.04] \ [110 - (0.088) \times 800)] - [0.054 - 0.088] \ [100 - (0.04 \times 800)]}{0.06419}$$

Simplifying:

$$y = \frac{[0.36 \times (110 - 70.4)] - [- 0.034 \times (100 - 32)]}{0.06419}$$

Thus:

$$y = \frac{(0.36 \times 39.6) - (-0.034 \times 68)}{0.06419} = \frac{14.256 + 2.312}{0.06419}$$

$$y = \frac{16.568}{0.06419} = 258.09 \text{ pounds of condensed milk}$$

$$m - x - y = \text{pounds of milk}$$

Substituting:

$$800 - 216.84 - 258.09 = 325.07 \text{ pounds of milk}$$

One difficulty may be encountered in proportioning or standardizing mixes by this or any other method. The operator may select materials whose composition make a desired combination impossible. Under such circumstances the value for x or y may be a minus quantity or their sum may be greater than the total amount of milk products possible in the mix.

REVIEW OF FOREIGN DAIRY LITERATURE

J. L. HILEMAN

Newark, N. J.

VIRTANEN. *On the Decomposition of Casein by Bact. casei and Strep. lactis.* (Societas Scientiarum Fennica Commentationes physico-mathematicae, Vol. 1, No. 41, p. 13, March, 1923. Abstracted in Le Lait, Vol. 5, August–September, 1925, p. 756.)

The author shows that Bact. casei ε, which cannot decompose casein at room temperature under ordinary conditions, can do so at 20° if the cultures have previously been maintained at 40° for twenty-four hours, which approximates the conditions of manufacture of Emmenthal cheese. He inoculated the organism into a half-liter of skim milk and added 30 grams of calcium carbonate. These cultures were maintained at 18° for one to two months, and were then analyzed for soluble nitrogen, amine nitrogen and ammoniacal nitrogen.

The Bact. casei ε died rapidly in the milk cultures containing the calcium carbonate, just as they do in cheese. However, Virtanen holds that they are still responsible for the decomposition of casein that takes place, this decomposition being due to an enzyme secreted by the organisms which continues to act after their death. From this the author argues that Bact. casei ε is the agent in the ripening of Emmenthal cheese, even though this cheese contains no Bact. casei ε after four to six months, but only Bact. casei α.

Strep. lactis, under the conditions found in the manufacture of Emmenthal cheese, also attacks casein. This organism and Bact. casei ε break down casein more vigorously when both are present than when only one is present, due to some symbiotic action.

GYORGY. *On the Distribution of Calcium and Inorganic Phosphorus in Milk.* (Biochemische Zeitschrift, Vol. 142, Nos. 1 and 2, October, 1923. Abstracted in Le Lait, Vol. 4, November, 1924, p. 779.)

This investigator attempts to answer the question as to whether or not there is a close relationship between the non-diffusible calcium phosphate and the casein in milk. He shows that the inorganic phosphates pass entirely into the serum obtained when milk is allowed to sour naturally, while only 40 per cent are found in the serum obtained when ren-

net acts on milk. That part of the phosphorus which will not pass through a Haen ultra-filter is 50 to 60 per cent in cow's milk, and 30 to 40 per cent in human milk.

At the iso-electric point of casein, all of the calcium and phosphorus are found dissolved in the serum. A solution of casein treated with lime and phosphoric acid gives the same results as milk.

These results are in close agreement with the work of Van Slyke and Bosworth at Geneva.

The author draws the conclusion that there is a close chemical relationship between the casein and the non-diffusible calcium phosphate of milk.

BARTHEL. *Influence of Molds on Lactic Ferments.* (Le Lait, Vol. 4, November, 1924, p. 725.)

Bacillus subtilis increases the activity of cultures of Streptococcus lactis. Certain molds also have such a symbiotic effect, among them being Mucor piriformis, Aspergillus niger and Penicillium glaucum. Oidium lactis appeared to have no effect.

CHARLES VAS. *Formation of Acid in Symbiosis by Bacillus bulgaricus and Streptocccus lactis in Yoghourt.* (Le Lait, Vol. 4, October, 1924, p. 625.)

Yoghourt is made by incubating milk containing a culture of Bacillus bulgaricus and Streptococcus lactis at 45°C. till coagulation occurs, and then reducing the temperature to 18° to 20°C. Under these conditions the mixed culture produces more acid, and produces it more quickly, than is produced by either organism is pure culture.

H. ROEDER. *Study of the Progress of Rennet Coagulation of Milk.* (Schweizerische Milchzeitung, No. 54, June, 1923. Abstracted in Le Lait, Vol. 4, November, 1924, p. 780.)

Roeder describes a unique apparatus for studying the rennet coagulation of milk, consisting of a piece of black glass moved by clockwork in such a way that it comes from the interior of the body of milk to the surface twice every second. The milk is placed in a vessel in a constant-temperature bath. The rennet is placed in a small boat floating on the surface of the liquid, and this is capsized by means of the thermometer used to observe the temperature of the milk at the required instant. The black glass coming periodically to the surface makes it possible to observe easily the appearance of the first floccules of curd, and to follow the course of the coagulation closely to completion.

Editor's Address Changed

Having accepted the position of head of the departments of dairy and animal husbandry at Massachusetts Agricultural College, effective April 1st, all manuscripts intended for the Journal of Dairy Science should be addressed to me c/o Massachusetts Agricultural College, Amherst, Mass.

J. H. FRANDSEN, *Editor*

WILLIAM ALONZO STOCKING, JR.

WILLIAM ALONZO STOCKING, JR.

William Alonzo Stocking, Jr., was born May 13, 1872, on a farm near the town of Simsbury, Connecticut. His parents were William A. and Serinda (Delanoy) Stocking. He married Harriet M. Bliss of Binghamton, N. Y., on June 27, 1900. The widow and their four children John, Robert, William and Elizabeth, all survive their husband and father, who died February 3, 1926, at his home on Cayuga Heights, Ithaca, N. Y.

Professor Stocking graduated in 1895 from the Connecticut Agricultural College (Storrs) with the degree B.Agr. and three years afterward received the B.S.A. degree at Cornell University. He acted as Instructor in Agriculture at the Mansfield (Penn.) Normal School during the academic year 1898–1899, after which he returned to the Connecticut Agricultural College as Assistant Professor and later as Professor of Dairy Bacteriology. He remained at Storrs until 1906 when he received an appointment as Assistant Professor of Dairy Bacteriology at the New York State College of Agriculture at Ithaca. Meanwhile (1904) he had received the M.S. degree from Cornell University.

In 1909 at the time when Professor R. A. Pearson left to become the Commissioner of Agriculture of New York State, he was given a full professorship at Cornell and was appointed head of the Department of Dairy Industry. During 1913 and 1914, after the resignation of former Dean Liberty Hyde Bailey, Professor Stocking was Acting Director of the College of Agriculture and of the Cornell Agricultural Experiment Station. He resigned his responsibility as head of the Department of Dairy Industry on June 14, 1923, continuing the duties of Professor of Dairy Bacteriology until November 1925, when his illness compelled him to stop work.

Such is the simple biographic and academic record of a man who has been one of the leading contributors to the scientific development of the dairy industry in the United States of America.

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Professor Stocking was one of the earliest workers in the field of dairy bacteriology to undertake the evaluation of the importance of the various sources of bacterial contamination of market milk. This work was done at Storrs. It included a survey of the results actually being obtained on a series of dairy farms and a study of the effect of covered pails, of feeding dusty feeds, and of cleaning cows, upon the bacteria count and dirt content of milk. Likewise, he was the first to make exhaustive study of the causes of the excessive contamination of milk where milking machines were used and through his efforts American manufacturers were aided in putting really successful milking machines on the market. His report on methods of sterilizing milking machine tubes contains the first detailed bacteriological report on the method of placing the tubes in brine, a method which today still is regarded as one of the most useful that has been suggested.

He likewise took part with Professors H. W. Conn and W. M. Esten in the pioneer study of the bacterial species found in dairy products, the final report on this work appearing in the 1906 Annual Report of the Storrs Station. He also shared in the studies of foreign type cheeses, particularly Camembert, which were undertaken under the leadership of Professor Conn.

A heavy burden of teaching and later of administrative responsibility fell upon Professor Stocking during his later years in Ithaca so that, to his deep regret, he was unable to maintain his investigational work. Many a young man felt his guiding hand and today his students are to be found in many places in the dairy industry where a scientific training is necessary for the greatest success. His influence will abide through the constructive work that these men are doing.

During this period he prepared the fine discussion of the types of bacteria found in dairy products which appeared as a section of Marshall's Microbiology, and wrote a "Manual of Milk Products" a book widely used as a text in agricultural colleges. His influence was widely felt throughout the dairy industry of the State and of the Nation. This influence was especially evidenced through his activities in connection with Farmers' Week programs, membership in the New York State Agricultural Society

and the New York State Dairymen's Association. He was particularly active in the latter association and served as its President from 1922 to 1924.

Professor Stocking was interested in the formation of the National group of dairy instructors into the Official Dairy Instructors' Association in 1906. He continued his active interest in this Association when it broadened its field of work and became the American Dairy Science Association. He served as President of this Association from 1916 to 1918, and as a member of many of its important committees. He was a member of the editorial board of the JOURNAL OF DAIRY SCIENCE from the time of its foundation by this Association in 1917.

The splendid dairy building of the College of Agriculture, dedicated at the time of the World's Dairy Congress in 1923, will remain as a fitting memorial to his untiring efforts for the advancement of dairy science in America. Upon his retirement from the administrative responsibilities of the Department of which he had been the head for so many years, he took particular delight in renewing his interest in the Society of American Bacteriologists of which he had been a member since 1912, attending the National meetings of this society in 1923 and 1924 and taking an active interest in maintaining the work of the Central New York Branch of this Society. In April 1924 Professor Stocking represented Cornell University and the United States Department of Agriculture at the International Dairy Exposition in Milan, Italy, and studied the dairy industry in Italy, Switzerland, France, Belgium, Holland and England.

In addition to his connection with the New York State College of Agriculture as a student, Professor Stocking served on the Staff through twenty years that are particularly significant because they constitute the period in which the broad policies of the institution have been cast. Professor Stocking was an active member of that earlier group that gave character and strength to a vigorously growing institution. To his special credit must be placed the development of dairy industry as a large and strong unit in the college organization. He will be sadly missed by his old associates, most of all because of the kindly spirit that pervaded all of his relationships.

In these days when there are many who fail to appreciate the fact that true science and true religion should work together harmoniously in their search after the truth that endures, it may be well to record also that Professor Stocking was active in the First Presbyterian Church of Ithaca, serving on the session of this church for eleven years. He was sincere in his love for his fellowmen and his purpose to help them, ever ready to go out of his way to do the thoughtful thing and say the gracious word. He bound men to him in abiding friendships. His spirit was broad and tolerant. He had profound convictions and held himself rigidly to them, but was ever charitable in his judgment of others. He gave the impression of singular gentleness, a life from itself set free. He leaves with those who came into contact with him only memories which strengthen faith in things which give hope and dignity to the meaning of human life.

E. S. GUTHRIE AND R. S. BREED.

THE COMPOSITION, DIGESTIBILITY AND FEEDING VALUE OF HYDROLYZED SAWDUST*

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This investigation was undertaken at the instigation and with the coöperation of the Forest Products Laboratory of the United States Department of Agriculture, located at Madison, Wisconsin. Sherrard and Blanco (1) of that laboratory have carried on extensive experiments in the conversion of the woody fiber of the sawdust from several species of wood, into a more soluble form for the production of industrial alcohol, and as a possible source of cattle food.

The method of treatment consists in cooking the sawdust under 120 pounds pressure with dilute sulphuric acid, which converts a portion of the cellulose and allied substances into sugar. The liquor resulting from the digestion together with the washings from the undigested sawdust residue is neutralized with lime and evaporated to a thick syrup, which is mixed with the dried residue. The product is then ready for feeding. It is a dark brown somewhat powdery meal with a slightly sweet woody odor and a woody flavor.

Its feeding value was investigated first by the Animal Husbandry Department of the Wisconsin Agricultural Experiment Station (2) and later by the Dairy Division, Bureau of Animal Industry, United States Department of Agriculture, at its experiment farm in Beltsville, Maryland (3). The results of these trials were somewhat conflicting, hence the request from the Forest Products Laboratory to this station to coöperate in carrying on further investigations.

Our work on the product has been divided into four phases:

1. Determination of its proximate composition
2. A preliminary palatability test
3. Determination of its digestibility
4. Its feeding value for milk production

TABLE 1

Proximate composition of hydrolyzed sawdust

LABORATORY NUMBER	MATERIAL	WATER AS RECEIVED	DRY MATTER BASIS				
			Ash	Protein	Fiber	N-free extract	Fat
728	Hydrolyzed sawdust (Douglas fir).........	6.80	2.04	0.05	48.73	47.65	1.53
733	Hydrolyzed sawdust (Douglas fir).........	7.43	2.24	0.00	48.68	47.83	1.25
740	Hydrolyzed sawdust (Douglas fir).........	7.09	2.75	0.15	46.63	49.40	1.07
769	Hydrolyzed sawdust (Douglas fir).........	6.33	2.45	0.80	46.21	49.29	1.25
790	Hydrolyzed sawdust (Douglas fir).........	7.45	3.42	0.15	42.82	52.13	1.49
830	Hydrolyzed sawdust (Douglas fir).........	7.50	2.39	0.74	45.43	48.94	2.50
842	Hydrolyzed sawdust (Douglas fir).........	7.40	2.55	0.31	46.44	49.31	1.38
856	Hydrolyzed sawdust (Douglas fir).........	7.33	2.56	0.12	45.03	50.15	2.15
Average.....................................		7.17	2.55	0.29	46.25	49.34	1.58
828	Hydrolyzed sawdust (Eastern white pine)	6.24	3.30	0.42	49.70	43.73	2.85
859	Hydrolyzed sawdust (Eastern white pine)	6.22	3.95	0.50	45.04	48.02	2.49
889	Hydrolyzed sawdust (Eastern white pine)	4.08	2.38	0.51	47.65	44.93	4.52
893	Hydrolyzed sawdust (Eastern white pine)	4.06	2.70	0.36	47.55	45.46	3.93
900	Hydrolyzed sawdust (Eastern white pine)	4.72	3.10	0.60	47.30	45.43	3.57
Average.....................................		5.06	3.09	0.48	47.45	45.51	3.47
862	Residue from hydrolyzed Douglas fir......	20.36	0.36	0.50	60.73	36.25	2.16
863	Residue from hydrolyzed Eastern white pine....................................	4.58	0.23	0.90	59.91	34.94	4.02
871	Residue from hydrolyzed Eastern white pine....................................	4.40	0.48	0.69	60.23	34.91	3.70
Average.....................................		4.49	0.36	0.80	60.07	34.93	3.86

PROXIMATE COMPOSITION

This was determined by the usual methods of fodder analysis.[1] Table 1 presents the detailed analyses.

As would be expected the product is almost entirely carbohydrate in nature, the crude fiber and nitrogen-free extract constituting on an average about 95 per cent of the total dry matter. Of the nitrogen-free extract, a considerable portion is sugar, according to Sherrard and Blanco (1) about 21 per cent of the original sawdust being converted into sugar by the process. The balance of the nitrogen-free extract is probably largely composed of lignin. The crude fiber is principally crude cellulose admixed with some lignin. The protein moiety is practically negligible, especially so in the case of the Douglas fir sawdust, while the fat is not true fat but resinous matter. The ash is chiefly an additive component, consisting for the most part of calcium sulphate formed by the neutralization with lime of the sulphuric acid used in the process. Laboratory Nos. 862, 863 and 871 are samples of the sawdust residue, with which, at the special request of this department, the syrup containing the sugar produced by the hydrolysis, was not mixed. The reason for investigating these residues is given in the section on digestibility (see p. 260). The main differences in composition between the residue and the complete hydrolyzed product are seen in a higher crude fiber content and a correspondingly lower amount of nitrogen-free extract, due to the removal of the sugar-holding liquor by centrifugation and washing. The ash content is that of the natural wood.[2]

PALATABILITY

The first step in the investigation was to determine if cows would eat the sawdust. Eleven animals in the station herd in groups of two at a time were offered the material as a supplement to their grain ration in amounts not exceeding 5 pounds daily, for periods varying with the different individuals from one to four months. Ten of the cows ate the sawdust, when moistened and mixed with their grain, with no apparent ill effects. Eight of them showed no dislike for it when fed in this way. All the cows refused it when it was fed alone, even when placed before

[2] For a more detailed study of the composition of the material the reader is referred to the work of Sherrard and Blanco.

them previous to the regular grain feeding. The Douglas fir sawdust was the kind used for this test. The maximum amount that a cow would consume without waste was 4 pounds daily.

Commencing in June, 1924, and ending in February, 1925, 6 digestion experiments with 3 sheep in each, i.e., 18 single digestion trials were carried on. Each of these experiments was of sixteen days duration and the materials investigated were: hydrolyzed Douglas fir sawdust, hydrolyzed Eastern white pine sawdust, and the residue from hydrolysis of Eastern white pine sawdust. The digestibility of this residue minus the liquor was determined to check up the assumption that it is probably without food value, the reasoning being that if it proved to be valueless why use it, or so much of it, as an absorbent for the liquor? Why not use as an absorbent some material having food value in itself?

A uniform ration was fed to the sheep throughout all the experiments. It consisted of:

Grams daily

```
Hay..........................................................400
Gluten feed..................................................125
Hydrolyzed sawdust...........................................100
Salt......................................................... 10
Water ad libitum
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The digestibility of the basal ration, consisting of hay and gluten feed was first determined by feeding these to the same sheep in a digestion trial identical with those in which the sawdust was added to the ration.

In all cases the sheep ate their daily allowance without waste, although they showed some reluctance about eating the *residue* from hydrolyzed Eastern white pine sawdust. No digestive disturbances were noted when the Douglas fir and Eastern white pine sawdust were fed, but when the *residue* from the white pine was fed there was marked disturbance as evidenced by the condition of the feces which became very soft and bulky.

Table 2 summarizes the results of the work.

DISCUSSION OF RESULTS

a. Douglas fir sawdust. Excluding from the average two

TABLE 2

Summary of digestion coefficients, expressed as percentages on a dry matter basis

	SHEEP	EXPERIMENT	DRY MATTER	FIBER	N-FREE EXTRACT
			Douglas fir sawdust		
I	23	2	31.49	15.09	59.30
II	23	4	48.48	17.63	76.85
III	24	2	35.46	6.76	62.07
IV	24	4	31.83	0.90	64.48
V	25	2	25.48	None	55.71
VI	25	4	32.13	0.52	54.99
Average..........................			34.15	Not averaged	62.23
Average omitting II and V........			32.73		60.21
			Eastern white pine sawdust		
I	23	3	46.69	18.72	65.46
II	23	5	44.05	10.87	72.37
III	24	3	44.42	21.03	64.49
IV	24	5	36.45	None	64.47
V	25	3	49.73	24.46	63.34
VI	25	5	27.66	None	52.54
Average..........................			41.50	Not averaged	63.78
Average omitting IV and VI			46.22		66.42
			Residue from hydrolysis of Eastern white pine sawdust.		
I	23	6	1.69	4.25	12.53
II	23	7	13.20	10.89	27.03
III	24	6	22.79	16.55	31.74
IV	24	7	None	None	0.45
V	25	6	32.41	20.58	37.61
VI	25	7	None	None	None
None of these results can be averaged					

results which deviate considerably from the mean, the dry matter
of this material had a digestibility of roughly 33 per cent, which

consisted almost entirely of nitrogen-free extract. The actual amount of nitrogen-free extract digested was equal to 28.5 pounds for each 100 pounds of sawdust (average of four single trials). This is a slight excess over the amount of sugar reported by Sherrard and Blanco (1) as being formed in the process of hydrolysis. It is evident that digestion was confined principally to this soluble carbohydrate, the other constituents being practically unattacked.

b. Eastern white pine sawdust. Excluding here also two deviating results, it is seen that this sawdust contained about 46 per cent of digestible dry matter, approximately 40 per cent more than the Douglas fir sawdust contained. The amount of nitrogen-free extract digested (28.6 pounds per 100) was almost identical with that digested in the case of the Douglas fir, so it is evident that some other proximate constituent must have been utilized to account for the higher total digestibility. Although the coefficients for fiber show a quite low digestibility (24.46 per cent as a maximum) they are somewhat higher than those obtained with the Douglas fir and we must assume that the difference in total digestible dry matter is due to a more digestible fiber in the pine, for by no stretch of either figures or imagination can the small amounts of protein, fat and ash present be made to account for it. The assumption is that in the pine wood the linkage between the cellulose and the lignin is less firm than in the Douglas fir, hence is more readily broken down or sprung apart by the treatment, thus giving the bacteria in the ruminant's digestive tract a better opportunity to act upon the cellulose molecule. It is evident from the wide variations in the digestion coefficients for fiber that the animals experienced difficulty in digesting material of this character.

c. Eastern white pine sawdust residue. A glance at the variable results obtained for this material is sufficient to show that its total digestible matter is so low that it can have no food value whatever. It simply proves the already well-grounded assumption that whatever food value the hydrolyzed sawdust may possess is contained largely in the liquor or syrup produced in the process of hydrolysis.

The white pine residue having given such poor results, it was deemed unnecessary to investigate the residue from Douglas fir, the entire hydrolyzed product from that wood having made a somewhat poorer showing than that from the pine.

Dismissing without further consideration the so-called "residue," it will be well to consider the entire hydrolyzed product from the standpoint of net energy. This station is not equipped for the direct determination of net energy in feeds. There is, however, a fairly reliable formula (4) by which the net energy value of a feed can be calculated if its digestible matter is known, and it is proposed to apply this formula here. Calculated in this manner, the Eastern white pine sawdust has an apparent net energy value of 18.6 therms per 100 pounds, about half that of average quality hay, while the net energy value of the Douglas fir sawdust is a minus quantity, or in other words, more energy is used up in the labor of digesting it than it contains itself.

<center>VALUE FOR MILK PRODUCTION</center>

This phase of the investigation was commenced in October, 1924, and was completed in July, 1925. The kinds of hydrolyzed sawdust fed were the same as used in the digestion studies, viz., Douglas fir and Eastern white pine.

Each kind of sawdust was submitted to an eleven weeks' feeding trial with 6 mature milch cows in the experiment station herd. Each of the eleven-week feeding periods was divided into two four-week periods of actual trial with a ten-day preliminary feeding in every instance, to accustom the cows to the change of feed.

The value of the sawdust for milk production was ascertained by comparison with corn starch, an equivalent amount of each from the standpoint of digestible nutrients being alternately substituted for a portion of each cow's grain for the length of time indicated above. Corn starch was chosen for comparison because it was the only material available which did not contain protein, of which there is none in the sawdust.

The herd was divided into two groups of three cows each. One group received the "starch" ration for thirty-eight days, while the other group received the "sawdust" ration. At the end of thirty-

eight days the feeds were reversed, those cows that had been getting starch being changed to sawdust and vice versa, for another period of thirty-eight days. Thus each and every cow received both rations. The last twenty-eight days of each period was taken as the basis for calculation of results. The Douglas fir sawdust was fed out first, its trial lasting from October 1, 1924, until December 14, 1924, all cows being in the experiment simultaneously. Because of delays in receipt of the white pine sawdust and also because of irregularity in the freshening dates of the cows available, not all the cows were in this experiment simultaneously. The feeding trial lasted from February 27, 1925, until July 7, 1925.

The ration fed to the cows consisted of: mixed hay and a grain mixture composed of corn meal, 20 per cent; wheat bran, 10 per cent; ground oats, 30 per cent; cottonseed meal, 10 per cent; linseed meal, 15 per cent, and gluten feed, 15 per cent. This mixture contained 16 per cent of digestible protein and constituted about 80 per cent of the daily grain allowance of each cow. The remaining 20 per cent was either starch or an equivalent amount of sawdust. The rations were adjusted in accordance with accepted standards at the beginning of each month, and were calculated a trifle scant in order to have the cows utilize the sawdust as fully as possible.

The amount of sawdust fed was the same in all cases, viz., 4 pounds daily, previous experience having shown that the cows would not readily eat any greater amount. The amount of starch equivalent to this amount of sawdust, taking into consideration the moisture content and relative digestibility of the starch and the two kinds of sawdust, was 1 pound and 4 ounces in the case of the Douglas fir sawdust, and 1 pound and 12 ounces in the case of the white pine sawdust. These were the amounts of starch fed.

The sawdust and starch were well mixed with the other grain before feeding to insure complete consumption. With one exception all the cows throughout both experiments ate their grain-sawdust mixture without waste. The one cow who was at times rather fussy wasted only a few pounds. No digestive disturbances were noted and both experiments were carried to completion satisfactorily.

In summarizing the results attention has been centered on the following points:

1. Milk yield
2. Inital shrinkage in milk flow
3. Change in body weight

MILK YIELD

In the experiment with Douglas fir sawdust versus starch 2 of the cows dried off much more rapidly than we had anticipated, and the same was true of 1 cow in the experiment with white pine sawdust versus starch. For this reason the milk yield records of these individuals have not been included in the reported results. Table 3 gives the comparative yield and quality of the milk in both experiments.

The cows produced 98.5 pounds less milk on the Douglas fir sawdust ration equal to about 4 per cent less solid matter than on the starch ration, and 42 pounds less milk, equivalent to substantially 1 per cent less solid matter on the white pine sawdust ration than on the starch ration. The figures point, therefore, to the fact that, on the basis of digestion equivalents, the hydrolyzed sawdust was rather inferior to the starch, and that the white pine product had a more favorable influence as a nutrient than did the Douglas fir. Differences in the percentage composition of the milk were so slight as to be without significance. The considerably higher percentages of total solids and fat in the Douglas fir experiment are due to the difference in stage of lactation, all the cows being rather advanced in lactation when on this trial, while all but one had recently freshened when put on the white pine sawdust.

INITIAL SHRINKAGE IN MILK FLOW

This was determined by noting the production of each cow during the week immediately preceding the commencement of the experiment and her production during the first week of the experiment proper, there being an interval of ten days between the two periods to allow for any irregularities due to change of feed. Table 4 presents the results.

TABLE 3
Milk production in the sawdust-starch feeding trial

DATE	COWS	MILK	SOLIDS		FAT	

Experiment I
Starch

		pounds	*per cent*	*pounds*	*per cent*	*pounds*
1924						
October 10 to	Colantha II	1,186.4	12.98	153.99	4.57	54.22
November 6	Colantha IV	609.8	12.16	74.15	3.75	22.87
November 17 to	Samantha IV	569.7	13.73	78.22	4.93	28.09
December 14	Fancy V	475.1	15.32	72.79	5.87	27.89
Total, pounds....................		2,841.0		379.15		133.07
Average, per cent................			13.35		4.68	

Sawdust (Douglas fir)

1924						
October 10 to	Samantha IV	691.9	12.87	89.05	4.48	31.00
November 6	Fancy V	504.1	15.23	76.77	5.93	29.89
November 17 to	Colantha II	1,123.0	13.10	147.11	4.46	50.09
December 14	Colantha IV	423.5	12.53	50.62	3.90	16.52
Total, pounds....................		2,742.5		363.55		127.50
Average, per cent................			13.26		4.65	

Experiment II
Starch

1925						
March 29 to April 25	Diantha	1,616.4	11.46	185.24	3.35	54.15
April 16 to May 13	No. 192	1,005.0	11.59	116.48	3.36	33.74
May 3 to 30	Colantha IV	1,419.0	11.90	168.86	3.62	51.36
May 6 to June 2	Samantha IV	1,238.8	12.24	151.63	4.02	49.80
June 10 to July 7	No. 46	1,046.5	10.96	114.70	3.03	31.71
Total, pounds....................		6,325.7		736.91		220.76
Average, per cent................			11.65		3.49	

Sawdust (Eastern white pine)

1925						
March 9 to April 5	No. 192	1,136.0	11.41	129.62	3.27	37.15
March 29 to April 25	Samantha IV	1,310.1	12.24	160.36	3.92	51.36
May 3 to 30	No. 46	1,108.0	11.10	122.99	3.25	36.01
May 6 to June 2	Diantha	1,465.3	11.62	170.27	3.54	51.87
June 10 to July 7	Colantha IV	1,264.3	11.67	147.54	3.47	43.87
Total, pounds....................		6,283.7		730.78		220.26
Average, per cent................			11.63		3.51	

TABLE 4

Showing relative shrinkage in milk yield when cows were put on the experimental ration—starch versus sawdust

COWS	MILK YIELD		INCREASE	DECREASE
	Production during week previous to commencement of experiment	Production during first week of experiment		

Experiment I
Starch

	pounds	pounds	pounds	pounds
Colantha II..........................	270.7	296.0	25.3	
Colantha IV..........................	183.9	160.8		23.1
Samantha IV........................	177.0	146.5		30.5
Fancy V............................	131.9	118.9		13.0
Total...........................	763.5	722.2	25.3	66.6

Sawdust (Douglas fir)

Samantha IV........................	223.8	172.2		51.6
Fancy V............................	137.7	121.1		16.6
Colantha II..........................	303.9	277.9		26.0
Colantha IV........................	139.6	106.0		33.6
Total...........................	805.0	677.2		127.8

Experiment II
Starch

Diantha............................	436.4	431.2		5.2
No. 192............................	278.0	260.2		17.8
Colantha IV........................	353.0	370.9	17.9	
Samantha IV........................	318.0	316.2		1.8
No. 46............................	255.7	253.6		2.1
Total...........................	1,641.1	1,632.1	17.9	26.9

Sawdust (Eastern white pine)

No. 192............................	297.5	288.4		9.1
Samantha IV........................	323.3	337.2	13.9	
No. 46............................	318.4	295.8		22.6
Diantha............................	381.5	378.8		2.7
Colantha IV........................	344.2	321.4		22.8
Total...........................	1,664.9	1,621.6	13.9	57.2

Note: The ten-day preliminary intervenes in each case between the two periods.

With the Douglas fir sawdust versus starch experiment the net decrease was: starch, 41.3 pounds; sawdust, 127.8 pounds. With the white pine sawdust it was starch, 9.0 pounds; sawdust, 43.3 pounds.

After the first month or so of lactation it is normal for a cow to gradually decline in milk production. Any increases reported in the table are due to the fact that the cows in question had not quite reached the peak of their production when the experiment was begun.

CHANGES IN BODY WEIGHT

All cows were weighed on each of three consecutive days at the beginning and end of each half of each experiment. The detailed data are shown in table 5.

TABLE 5

Gain or loss in weight in sawdust-starch experiment

Experiment I (Douglas fir sawdust)

COWS		STARCH			COWS		SAWDUST (DOUGLAS FIR)		
		Weight	Gain	Loss			Weight	Gain	Loss
No. 46............	B	1,265			Diantha.........	B	1,282		
	E	1,310	45			E	1,310	28	
Colantha II.......	B	1,090			Samantha IV.....	B	1,272		
	E	1,108	18			E	1,308	36	
Colantha IV.......	B	1,250			Fancy V..........	B	892		
	E	1,270	20			E	900	8	
Diantha..........	B	1,320			No. 46............	B	1,320		
	E	1,362	42			E	1,348	28	
Samantha IV......	B	1,300			Colantha II.......	B	1,145		
	E	1,365	65			E	1,133		12
Fancy V..........	B	915			Colantha IV......	B	1,300		
	E	910		5		E	1,298		2
Total......................			190	5				100	14

Starch—net gain...185 pounds
Sawdust—net gain.. 86 pounds

TABLE 5—*Continued.*

Experiment II (Eastern white pine sawdust)

COWS		STARCH			COWS		SAWDUST (EASTERN WHITE PINE)		
		Weight	Gain	Loss			Weight	Gain	Loss
Colantha II	B	1,170			No. 192	B	1,255		
	E	1,195	25			E	1,265	10	
Diantha	B	1,265			Samantha IV	B	1,265		
	E	1,285	20			E	1,290	25	
Colantha IV	B	1,245			No. 46	B	1,215		
	E	1,240		5		E	1,190		25
No. 192	B	1,280			Colantha II	B	1,240		
	E	1,325	45			E	1,250	10	
Samantha IV	B	1,275			Diantha	B	1,285		
	E	1,285	10			E	1,280		5
No. 46	B	1,200			Colantha IV	B	1,215		
	E	1,220	20			E	1,245	30	
Total			120	5				75	30

Starch—net gain..115 pounds
Sawdust—net gain.. 45 pounds

B = beginning of period; E = end of period.

Each weight reported above is an average of three weights taken on consecutive days.

CONCLUSIONS ON THE MILK PRODUCTION INVESTIGATION

The milk yield was slightly higher when starch was fed than when the digestion equivalent of either Douglas fir or white pine sawdust was fed. The white pine made a slightly better showing in this respect than did the Douglas fir.

The initial shrinkage in milk flow was much more marked with both sawdusts than with the starch.

Net gains in body weight were somewhat higher on the starch than on either sawdust although changes in weight were not pronounced.

It is not advisable in an experiment of this kind, with a relatively small number of cows, to draw too definite conclusions.

The results indicate, however, that the animals derived some benefit from the sawdust.

GENERAL CONCLUSIONS

As a result of our studies the following general conclusions are drawn:

1. Hydrolyzed sawdust is composed almost entirely of crude cellulose, lignin, and a mixture of hexose and pentose sugars.

2. Animals will not eat the hydrolyzed sawdust when fed by itself. In order to promote consumption it is necessary to mix it with other grains. Occasionally an animal will refuse to eat the mixture of which the sawdust is a component.

3. About 4 pounds daily is all that the mature dairy cow will consume, especially, if it is fed for any length of time.

4. Digestion studies show that the dry matter of the Eastern white pine sawdust was about 46 per cent digestible, while that of the Douglas fir sawdust was about 33 per cent digestible. In the case of the Douglas fir sawdust, digestion was confined principally to the invert sugar formed by the hydrolysis, while in the case of the white pine sawdust apparently some of the cellulose was digested also. The "residue" from the white pine sawdust is substantially worthless as a feed, and presumably the same is true of that from Douglas fir. Although no digestion studies were made on the latter the inference is that the total product from the Douglas fir having proved inferior to that from the white pine, the same would be true of the "residue."

5. If the process of hydrolysis could be modified so as to convert a larger proportion of the cellulose of the wood into sugar, or more completely separate the cellulose from the lignin the food value of the material would be enhanced. If the above is not possible from the standpoint of economy it might be worth while to mix the extract with a carrier having some substantial food value. The mixing of the extract with the inert residue produces an inferior food product.

6. The evidence at hand indicates that the white pine sawdust is likely to respond to treatment better than the Douglas fir.

This may be due to the fact that in the white pine the linkage between lignin and cellulose is less firm than it is in the Douglas fir.

7. On the basis of equal amounts of digestible nutrients the sawdust when fed to dairy cows produced only slightly smaller amounts of milk than did corn starch, but it took, on an average, 2.75 pounds of sawdust to equal one pound of starch.

8. On the basis of the present supply and cost of carbohydrate concentrates it is not believed that the product as now prepared has any economic value. Under unusual conditions, as in case of an extreme shortage of ordinary feedstuffs, it might be used as a partial substitute for the cereal grains or starchy by-products.

9. The Forest Service of the United States Department of Agriculture is to be commended for its efforts to find a use for the vast amounts of waste sawdust. Progress has been made and it is hoped that a continuance of the study will bring us nearer a satisfactory solution of the problem.

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PEROXIDASE AS A FACTOR IN BUTTER DETERIORATION*

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The extent to which the natural enzymes of milk contribute to the deterioration of butter is still an unsettled question. This is especially true for peroxidase, which is certainly the most abundant as well as the most potent of the oxidizing ferments normal to milk.

When cream is pasteurized at temperatures sufficiently high to destroy peroxidase, the keeping quality of the butter is often enhanced. Rogers, Berg, and Davis (1) Rogers, Berg, Potteiger, and Davis (2), Larson, Fuller, Jones, Gregory, and Tolstrup (3), and Hunziker, Spitzer, Mills and Switzer (4) have obtained results of this character, although Mortensen, Gaessler, and Cooper (5) were unable to detect such an effect. The data of Palmer and Combs (6) on tallowy decomposition of butter can also be interpreted as showing that the natural oxidases are a factor, at least in this type of deterioration.

Regardless of whether there are sufficient data to show definitely that the temperature of pasteurization is a major factor in determining the keeping quality of butter, it must be admitted that there has been a tacit assumption among buttermakers that the destruction of all enzymes in cream is an important aid in butter storage. So far as peroxidase is concerned the widespread use of the Storch test in creamery practice has given rise to the belief that peroxidase itself is detrimental to the keeping quality of butter.

A more direct means of determining the effect of peroxidase in butter deterioration suggested itself to us, namely, to add large quantities of the enzyme to cream which had been rendered free of enzymes by heat and to observe the keeping quality of the butter in comparison with check lots containing no peroxidase. The present paper sets forth briefly the results of such an experiment.

<div align="center">EXPERIMENTAL</div>

Method of procedure

Peroxidase was concentrated from horse-radish roots according to the method of Willstätter and Stoll (7) as modified by Willstätter (8). The aqueous solution of nearly pure peroxidase obtained from 10 kgm. of roots was concentrated under reduced pressure. This solution had a yellow color and spicy flavor. A qualitative examination showed that purpurogallin was readily produced in large quantities from pyrogallol when the enzyme solution was tested by a method essentially like that recommended by Rice and Hanzawa (9).

The effect of the enzyme was determined in both sweet and ripened cream butter at room temperature and at 0°C. A 40-pound lot of 40 per cent cream was pasteurized at 180°F. for ten minutes and then divided into four equal parts. Two of these were ripened with starter to an acidity of 0.6 per cent. One hundred ten cubic centimeters of peroxidase solution were added to one 10 pound lot of both sweet and ripened cream, the other two portions being kept as checks. All four lots were then churned and the butter drained, washed, salted, and worked in the usual manner. The butter from each lot was packed in one-pint glass jars having glass tops, rubber seals, and metal clamps. Aseptic conditions were maintained as far as possible throughout, all utensils, jars, etc., being sterilized in flowing steam. One-half of each lot of butter was stored at room temperature and the other half at 0°C. The average composition of the butter from 24 one-pint samples, including six from each lot, as determined by the Kohman method was as follows: fat, 82.5 per cent; moisture, 13.9 per cent; salt, 2.9 per cent; and curd, 0.7 per cent.

Examination of the various lots and sub-lots of butter was made at intervals of approximately 115, 180, and 335 days from the date of churning. The examination included (a) a study of the organic and amino acids in the aqueous phase of the butter, (b) peroxidase tests on the aqueous phase of the butter, (c) the Kreis test on the butter fat and (d) a determination of the amount of oxidized fatty acids in the fat. In addition there was a general examination for quality of the stored samples.

The organic and amino acids in the butter serum were determined by the Foreman (10) method, the serum being isolated from 200 grams of butter by a modification of the method suggested by Hunziker and others (4), the serum and washings being made up to 500 cc. volume. The presence of peroxidase was detected with the guaiac, paraphenylenediamine (Storch) and pyrogallol reagents. The Kreis test was made by Kerr's (11) modification and the amount of oxidized (petroleum-ether insoluble) fatty acids determined by Fahrion's (12) method.

Results

The effects of the added peroxidase were entirely negative throughout so far as any chemical changes or evidences of deterioration due to the presence of excessive amounts of peroxidase were concerned, making it unnecessary to give analytical results in detail. There was no proteolysis whatever in either the enzyme or check samples, even after eleven months at room temperature. It was not expected that peroxidase would be a factor in deterioration of this type; the result, however, serves to show that the butter was entirely free from other important agents of deterioration. All enzyme samples gave strong tests for peroxidase throughout the entire period of observation showing that active enzyme was present at all times. The Kreis test was negative for all samples, even those stored for nearly a year at room temperature. Petroleum-ether insoluble fatty acids were determined only at the end of the three months storage period. The amount ranged from 0.3 to 0.9 gram per 100 grams of butter fat, but there were no differences between the enzyme and check samples that could be determined by this method.

The general quality of all the samples of butter was good. There was no difference between the enzyme and check samples as to odor, flavor or color.   There was no bleaching in any case. All the samples stored at 0°C. were edible and were pronounced good butter, even after eleven months storage.   The samples held at room temperature for the same period were also remarkably well preserved considering their age and method of storage. All persons to whom they were shown exhibited great surprise at their quality.   The only pronounced defects in the cold storage and room temperature samples was a mealy quality in the former and a stale flavor in the latter.

CONCLUSIONS

Peroxidase in itself is not a factor in the deterioration of storage butter.   The enhanced keeping quality observed in butter made from cream pasteurized at high temperatures is due to the elimination of agents for deterioration other than the peroxidase enzyme.

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A BABCOCK-GERBER METHOD FOR DETERMINING THE PERCENTAGE OF FAT IN ICE CREAM*

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A fat standard for ice cream has been set by the Federal Government and by practically every state in the Union which makes a very eminent demand for a quick, inexpensive and fairly accurate method for determining the percentage of fat in ice cream. In the last few years there has been submitted to the dairy industry, numerous methods for determining the percentage of fat in the ice cream. The most recent of these methods is the Troy-Fucoma method which is a modification of the Gerber method of testing dairy products as used in Europe. Where careful analytical results are desired the Mojonnier method is to be recommended but this method takes 'time and is rather expensive when the cost of equipment is considered. Due to the cost of the Mojonnier test it is impracticable and impossible for the small ice cream manufacturer to use this method. Experiments with the Troy-Fucoma method have shown that this method checks closer with the Mojonnier than any of the other methods which are now in use for testing ice cream for butterfat. The belief that the reagents used in the Troy-Fucoma test would work in the Babcock test bottle and thus eliminate the necessity of buying additional testing equipment, led to this experiment.

PLAN OF EXPERIMENT AND EXPERIMENTAL METHODS

Forty samples of ice cream were tested by each of the three methods. These samples of ice cream were secured from the retail stores in New Brunswick and represent samples from six different manufacturers, a pint of ice cream being purchased for each sample. The samples were melted in a water bath and

brought to about room temperature. As soon as the samples had reached this temperature they were thoroughly mixed and charges weighed out for each of the three methods studied.

A. Babcock-Gerber method. Since amyl alcohol is slightly soluble in butterfat in the preliminary trials with this method the alcohol was used in the following amounts: 1.5, 1.8, and 2 cc. It was found that 1.5 cc. of amyl alcohol did not give as clear and as well defined fat columns or did the results check as close with the Mojonnier as when 1.8 or 2 cc. were used. In the Troy-Fucoma method they use 1 cc. of amyl alcohol per 5 grams of ice cream which is equivalent to using 1.8 cc. of alcohol per 9 grams of ice cream in the Babcock-Gerber method. In a few cases slightly higher results were secured by using 2 cc. of amyl alcohol. However, these results were well within the experimental error and for actual practice the solubility of the amyl alcohol in butterfat is not great enough to be a factor in the test when only enough is used to secure a clear fat column. Both the 9-gram 20 per cent ice cream bottles and the 9-gram 50 per cent cream bottles were used in this experiment. Since the 9-gram 20 per cent ice cream bottles are graduated in 0.2 per cent they should be used with this method. The samples were centrifuged in an electric non-heated tester. The directions for making the test are as follows:

1. Weigh exactly 9 grams of well mixed sample into 9 gram 20 per cent ice cream test bottle.

2. Add 9 cc. of water and 1.8 cc. of amyl alcohol.

3. Mix thoroughly.

4. Add 17.5 cc. of diluted commercial sulphuric acid (94 cc. of acid to 6 cc. of water for chocolate ice cream or 87 cc. of acid to 13 cc. of water for other flavors) in three proportions and mix thoroughly after each addition of acid and finally give samples a vigorous shaking. (To obtain a clear fat column this is very important.)

5. Centrifuge for five minutes.

6. Add hot water to bring contents up to the bottom of the neck of the bottle.

7. Centrifuge for two minutes.

8. Add hot water to bring the bottom of the fat column well within the neck of the bottle.

9. Centrifuge for one minute.

10. Place bottles in a hot water bath at a temperature of 135° to 140°F. and leave for five minutes.

11. Remove each bottle as it is to be read, add red reader and read per cent of fat with dividers.

B. Gerber or Troy-Fucoma method. Much difficulty was experienced with the Troy-Fucoma test in the preliminary trials and considerable time was spent in determining what method of procedure gave the best results. Troy's method as published by the Fucoma Company was first used and the results were very unsatisfactory both from standpoint of clearness of fat column and comparison to Mojonnier. The following method which is a slight modification of the one recommended by Fisher and Walts (1) was found to give the best results.

1. Measure 10 cc. of diluted commercial sulphuric acid (94 cc. of acid to 6 cc. of water for chocolate ice cream or 87 cc. of acid to 13 cc. of water for other flavors) into special ice cream test bottles.

2. Weigh exactly 5 grams of well mixed sample into test bottle using care to let the ice cream run down the side of the test bottle and spread over the surface of the acid without mixing it.

3. Add 5.5 to 6 cc. of water (depending on size of bottle) and 1 cc. of amyl alcohol; insert rubber stopper and mix contents by inverting test bottles until contents are thoroughly mixed.

4. Centrifuge immediately at a speed of 1000 to 1200 revolutions per minute for about four minutes.

5. Place in water bath at 135° and hold for about five minutes.

6. Centrifuge for two minutes.

7. As soon as centrifuging is complete place in water bath at 135°F. for five minutes and read with dividers.

C. Mojonnier method. The directions for testing ice cream for fat and total solids by the Mojonnier method as outlined by Mojonnier and Troy (11) were followed exactly.

RESULTS

The percentage of fat obtained on the forty samples by each of the three methods used and the total solid content are tabulated in tables 1, 2 and 3. Table 1 consists of eighteen samples of

vanilla, table 2 consists of twelve samples of chocolate and table 3 consists of ten samples of strawberry. Taking the Mojonnier or modified Roese-Gottleib method as the standard for comparison, the per cent of variation of each of the Troy-Fucoma and the Babcock-Gerber from the Mojonnier are reported in tables 1 to 3.

TABLE 1

Percentages of fat in vanilla ice cream as determined by Mojonnier, the Troy-Fucoma and Babcock-Gerber method

SAMPLE NUMBER	MOJONNIER		TROY-FUCOMA		BABCOCK-GERBER				PER CENT VARIATION		
					9 gram 20 per cent bottle		9 gram 50 per cent bottle		Troy-Fucoma from Mojonnier	Babcock-Gerber from Mojonnier	
	Fat	Total solids	Original	Duplicate	Original	Duplicate	Original	Duplicate		20 per cent bottle	50 per cent bottle
1	10.71	38.29	10.6	10.5	10.6	10.4	11.0	11.0	−0.16	−0.21	+0.29
2	11.19	39.86	10.8	11.0	11.4	11.4	11.5	11.5	−0.29	+0.21	+0.31
3	10.68	36.43	10.8	10.8	10.8	10.8	11.0	11.0	+0.12	+0.12	+0.32
4	11.65	37.78	11.2	11.6	11.8	11.8	12.0	12.0	−0.25	+0.15	+0.35
5	11.63	37.67	12.0	11.6	11.8	11.8	12.0	11.5	+0.17	+0.17	+0.12
6	14.64	40.35	13.8	13.8	14.5	14.4	14.5	15.0	−0.84	−0.19	−0.64
7	14.14	39.03	13.2	13.2	13.8	13.6	13.5	13.5	−0.84	−0.44	−0.64
8	14.59	40.52	14.4	14.6	14.8	14.6	15.0	15.0	−0.09	+0.11	+0.41
9	12.90	38.42	12.6	12.4	12.8	12.6	12.5	12.5	−0.40	−0.20	−0.40
10	14.05	38.67	13.6	13 4	14.2	14.2	14.0	14.0	−0.55	+0.15	−0.05
11	11.37	38.73	10.6	10.8	11.4	11.4	11.5	11.5	−0.61	−0.41	−0.31
12	12.92	37.85	12.6	12.8	12.8	12.8	13.0	13.0	−0.22	−0.12	+0.08
13	10.84	37.65	10.4	10.6	11.0	11.0	11.0	11.0	−0.34	+0.16	+0.16
14	11.56	39.09	12.6	12.6	11.6	11.4	11.5	12.0	+0.94	−0.06	+0.19
15	11.87	38.28	11.6	11.6	11.8	11.8	12.0	12.0	−0.27	−0.07	+0.13
16	11.07	38.53	10.6	10.4	11.0	11.2	11.0	11.0	−0.57	+0.03	−0.07
17	11.48	39.47	10.4	10.6	11.8	11.6	12.0	12.0	−1.02	+0.22	+0.52
18	11.04	38.23	11.2	11.4	11.2	11.2	11.0	11.0	+0.26	+0.16	−0.04
Average variation									0.44	0.17	0.25

The average variation from the Mojonnier on eighteen samples of vanilla ice cream was 0.44 per cent, in case of the Troy-Fucoma; 0.17 per cent, for the Babcock-Gerber method using the 9-gram 20 per cent ice cream bottles and 0.25 per cent using the 9-gram 50 per cent cream bottles. The results obtained with the chocolate samples were higher with both the Troy-Fucoma and Babcock-Gerber with the exception of the Babcock-Gerber when

TABLE 2

Percentage of fat in chocolate ice cream as determined by Mojonnier, the Troy-Fucoma and Babcock-Gerber method

SAMPLE NUMBER	MOJONNIER		TROY-FUCOMA		BABCOCK-GERBER				PER CENT VARIATION		
					9 gram 20 per cent bottle		9 gram 50 per cent bottle		Troy-Fucoma from Mojonnier	Babcock-Gerber from Mojonnier	
	Fat	Total solids	Original	Duplicate	Original	Duplicate	Original	Duplicate		20 per cent bottle	50 per cent bottle
1	12.16	41.91	11.8	11.7	12.4	12.3	12.5	12.5	−0.41	+0.19	+0.34
2	10.99	38.81	11.0	10.7	11.0	11.0	11.0	11.0	−0.14	+0.01	+0.01
3	16.44	44.40	16.0	16.2	16.4	16.5	16.5	16.5	−0.34	+0.01	+0.06
4	15.53	43.38	14.6	15.0	15.4	15.5	15.5	15.5	−0.73	−0.08	−0.03
5	14.18	41.57	13.6	13.2	13.6	13.6	13.5	13.5	−0.78	−0.58	−0.68
6	15.00	40.64	14.0	14.0	14.4	14.6	14.5	14.5	−1.00	−0.50	−0.50
7	11.72	40.26	11.4	11.2	11.8	11.8	12.0	12.0	−0.42	+0.08	+0.28
8	11.81	40.18	11.2	11.2	11.4	11.4	11.5	11.5	−0.36	+0.04	+0.24
9	10.70	39.82	9.6	9.8	10.2	10.2	10.5	10.5	−1.00	−0.70	−0.20
10	11.53	39.86	11.4	11.4	11.6	11.6	11.5	11.5	−0.13	+0.07	−0.03
11	10.86	40.45	10.4	10.4	10.4	10.4	10.5	10.5	−0.46	−0.46	−0.36
12	11.36	41.04	11.0	11.2	11.6	11.4	11.5	11.5	−0.25	+0.15	+0.15
Average variation									0.51	0.24	0.24

TABLE 3

Percentage of fat in strawberry ice cream as determined by Mojonnier, the Troy-Fucoma and Babcock-Gerber method

SAMPLE NUMBER	MOJONNIER		TROY-FUCOMA		BABCOCK-GERBER				PER CENT VARIATION		
					9 gram 20 per cent bottle		9 gram 50 per cent bottle		Troy-Fucoma from Mojonnier	Babcock-Gerber from Mojonnier	
	Fat	Total solids	Original	Duplicate	Original	Duplicate	Original	Duplicate		20 per cent bottle	50 per cent bottle
1	9.61	39.67	9.8	9.8	9.8	9.8	10.0	10.0	−0.19	+0.19	+0.39
2	13.79	40.62	13.8	14.0	14.0	14.0	14.0	14.0	+0.11	+0.21	+0.21
3	12.36	40.68	11.8	12.0	12.2	12.2	12.0	12.0	−1.15	−0.16	−0.36
4	12.97	38.47	13.0	13.0	13.0	13.0	13.0	13.0	−0.03	−0.03	−0.03
5	9.51	37.65	9.4	9.6	9.6	9.4	9.5	9.5	+0.01	+0.01	+0.01
6	10.26	38.10	10.0	9.8	10.2	10.4	10.5	10.5	−0.36	+0.04	+0.24
7	10.51	34.73	9.8	9.8	10.2	10.2	10.5	10.5	−0.71	−0.31	−0.01
8	9.63	37.20	10.0	9.8	10.0	9.8	10.0	10.0	−0.27	−0.27	−0.37
9	9.72	37.75	9.8	10.0	10.0	10.0	10.0	10.0	+0.18	+0.28	+0.28
10	9.44	38.23	8.6	8.8	9.4	9.4	9.5	9.5	−0.74	−0.04	+0.06
Average variation									0.37	0.15	0.19

the 9-gram 50 per cent test bottles were used. The average variation from the Mojonnier in the case of the twelve chocolate samples was 0.51 per cent for Troy-Fucoma and 0.24 per cent for Babcock-Gerber for both kinds of test bottles. The reason for these higher results is likely due to the fact that chocolate ice cream is generally believed to be much harder to test by any method than the other flavors because of the rich fat content in the chocolate itself, and that the acid does not completely dissolve the solids not fat. The strawberry ice cream checked closer with the Mojonnier by both methods than did either of the other two flavors. The reason for this may be that on the average the fat and total solids content of the strawberry ice cream was lower than the other two flavors. The difference, when compared to the chocolate samples, amounts to about 2 per cent for fat and 2.7 per cent for total solids. The average variation from the Mojonnier on ten samples of strawberry ice cream was 0.37 per cent in case of Troy-Fucoma; 0.15 per cent for the Babcock-Gerber using the 9-gram 20 per cent ice cream bottles and 0.19 per cent using the 9-gram 50 per cent cream bottles.

Considering the three flavors as one group the average variation from the Mojonnier on the forty samples was 0.44 per cent in case of Troy-Fucoma, 0.19 per cent for the Babcock-Gerber using the 9-gram 20 per cent ice cream bottles and 0.23 per cent using the 9-gram 50 per cent cream bottles. The average variation for the Troy-Fucoma method is slightly higher than that found by Fisher and Walts (3) and the variation for the Babcock-Gerber method is much less than the one they reported for their Modified Babcock method using ethyl alcohol and sulphuric acid.

In order to determine the accuracy of the Troy-Fucoma and Babcock-Gerber methods when the Mojonnier is used as a standard for comparison, the results of the forty samples have been summarized in table 4.

A study of this table shows more favorable results for the Babcock-Gerber method. In the case of this method 67.5 per cent of all samples checked within 0.2 per cent (smallest graduation on bottle), while with the Troy-Fucoma method only 25

per cent of samples checked within 0.2 per cent of the Mojonnier. If a comparison is made with the Babcock-Gerber method using the 9-gram 50 per cent cream bottles, twice as many samples checked within 0.2 per cent of Mojonnier as in case of Troy-Fucoma and 92.5 per cent checked within 0.5 per cent (smallest graduation on bottle). Also only 38 samples or 95 per cent of

TABLE 4

Summary of percentages of variation of Troy-Fucoma from Mojonnier method

 3 samples or   7.5 per cent checked within 0.1 of 1 per cent of Mojonnier
10 samples or 25.0 per cent checked within 0.2 of 1 per cent of Mojonnier
18 samples or 45.0 per cent checked within 0.3 of 1 per cent of Mojonnier
22 samples or 55.0 per cent checked within 0.4 of 1 per cent of Mojonnier
25 samples or 62.5 per cent checked within 0.5 of 1 per cent of Mojonnier
32 samples or 80.0 per cent checked within 0.75 of 1 per cent of Mojonnier
38 samples or 95.0 per cent checked within 1.00 of 1 per cent of Mojonnier

*Summary of percentages of variation of Babcock-Gerber from Mojonnier method
using 9 gram 20 per cent ice cream bottle*

13 samples or 32.5 per cent checked within 0.1 of 1 per cent of Mojonnier
27 samples or 67.5 per cent checked within 0.2 of 1 per cent of Mojonnier
33 samples or 82.5 per cent checked within 0.3 of 1 per cent of Mojonnier
34 samples or 85.0 per cent checked within 0.4 of 1 per cent of Mojonnier
38 samples or 95.0 per cent checked within 0.5 of 1 per cent of Mojonnier
40 samples or 100.0 per cent checked within 0.75 of 1 per cent of Mojonnier

*Summary of percentages of variation of Babcock-Gerber from Mojonnier method
using 9 gram 50 per cent cream bottle*

12 samples or 30.0 per cent checked within 0.1 of 1 per cent of Mojonnier
20 samples or 50.0 per cent checked within 0.2 of 1 per cent of Mojonnier
24 samples or 60.0 per cent checked within 0.3 of 1 per cent of Mojonnier
34 samples or 85.0 per cent checked within 0.4 of 1 per cent of Mojonnier
37 samples or 92.5 per cent checked within 0.5 of 1 per cent of Mojonnier
40 samples or 100.0 per cent checked within 0.75 of 1 per cent of Mojonnier

all samples checked within 1.00 per cent from Mojonnier in case of Troy-Fucoma and all the samples checked within 0.75 per cent in the case of Babcock-Gerber.

A comparison was made as to whether these methods gave results which were higher or lower than the Mojonnier and the study showed 32 out of the 40 samples gave results somewhat lower than the Mojonnier in case of Troy-Fucoma method, while

only 18 samples were lower with the Babcock-Gerber method for the 9-gram 20 per cent ice cream bottles and 17 samples for the 9-gram 50 per cent cream bottles.

Much difficulty was experienced with the Troy-Fucoma method in securing a clear fat column and also duplicate checks. If the method used was such that the fat column consisted of about half fat and the other half a hazy film the results seemed to check closer but the difficulty with this is to secure the right amount of hazy film. Also by screwing the rubber stopper at the bottom of the bottle some of the materials are forced up into the fat column thus increasing the reading. In this experiment duplicate determinations were repeated until a clear fat column was

TABLE 5

Effect of total solids on percentages of variations of Troy-Fucoma and Babcock-Gerber methods from Mojonnier method

	TROY-FUCOMA	BABCOCK-GERBER	
		20 per cent bottles	50 per cent bottles
Average variation of all 40 samples...............	0.44	0.19	0.23
Average variation of all 20 samples with highest solid content...................................	0.58	0.23	0.30
Average variation of 20 samples with lowest solid content..	0.32	0.14	0.16

obtained and in some cases with chocolate ice cream this required as many as six trials. In the case of one sample of chocolate ice cream not reported in this experiment a total of seven trials was made and on only one bottle was a fat reading of 10.6 per cent secured and the reading on duplicate bottle was less than 9 per cent. The Mojonnier on this sample was 12.05 per cent and the Babcock-Gerber 11.85 for the 9-gram 20 per cent ice cream bottles and 12 per cent for the 9-gram 50 per cent cream bottles. In no case was more than one determination required on any one sample with the Babcock-Gerber method.

A study of accuracy of the Troy-Fucoma and Babcock-Gerber methods as effected by the total solids in the ice cream are reported in table 5.

From this table it will be noted that the 20 samples with lowest solid content checked closer by both methods. All samples in the second group have a solid content above 39 per cent. Since these figures show a wide variation, similar experiments should be conducted at other laboratories, before any definite conclusions should be drawn.

SUMMARY AND CONCLUSIONS

1. The Babcock-Gerber method for determining the percentage of fat in ice cream is the adaption of the reagents in the Troy-Fucoma method applied to Babcock equipment.

2. To study the relative value of the Babcock-Gerber method as compared to the Troy-Fucoma and Mojonnier method, 40 samples of ice cream were analyzed by these three methods.

3. Much difficulty was experienced in obtaining clear fat columns and duplicate checks with the Troy-Fucoma method.

4. Using the Mojonnier method as a standard for comparison the results indicate that the Babcock-Gerber method is 231 per cent more accurate than the Troy-Fucoma.

5. The average per cent of variation from the Mojonnier on 40 samples was 0.44 per cent for Troy-Fucoma, 0.19 per cent for Babcock-Gerber using the 9-gram 20 per cent ice cream bottles and 0.23 per cent using the 9-gram 50 per cent cream bottles.

6. The Babcock-Gerber method seems well adapted for factory use where simplicity, cost of equipment and time are important considerations, however, where accurate analytical results are desired the Mojonnier should be used.

7. With the Babcock-Gerber method 67.5 per cent of all samples checked within 0.2 per cent while with the Troy-Fucoma method only 25 per cent of the samples checked within 0.2 per cent of the Mojonnier.

8. It would seem that the higher the total solids content, the more difficult it is to obtain accurate results when sulphuric acid is used as the solvent.

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OFFICIAL RECORDS AS MATERIAL FOR STUDYING INHERITANCE OF MILK AND BUTTERFAT PRODUCTION*

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During the past two decades a large number of purebred cows of the dairy breeds have been officially tested for milk and butterfat production. These tests have been carefully supervised by the agricultural colleges of the country, and the accuracy and thoroughness of this work is safeguarded by carefully drawn and rigidly enforced rules. The results of these tests as published by the breed associations in the various volumes of the Advanced Register and Register of Merit make up a vast reservoir of material of recognized value for the statistician.

For the purpose of assaying these records as material for studying the inheritance of milk and butterfat production, the initial and reëntry records of all cows of three breeds having two or more records have been arranged in age groups. This method of study was used in order to determine the correlation existing between records made at two different ages by the same group of cows. The correlations are based on actual initial and reëntry records made at corresponding ages by each cow in the groups.

It is assumed that any official record of production is a demonstration of the inherited ability of the cow to produce milk and butterfat, and the extent to which this ability is hindered by the combination of things constituting environment. The term environment is used in its broad sense, and includes all factors other than genetic or heritable factors, such as methods of feeding, climate, housing, number of daily milkings, season of freshening, disease, accidents, etc. If we assume that the environment is such as to afford the inherited ability full and unrestricted play; then it would appear that the correlation between the initial and

reëntry records made by a group of cows at corresponding ages should be perfect.   In other words, the relative ranking of the records of a group of cows on reëntry should be the same as the relative ranking of the initial records made by the same group of cows at an earlier age.   The variation of this correlation from plus one should measure the extent to which environment interferes with the complete expression of the inherited ability, and should indicate the value of such records for studying heredity.

Nineteen groups of Guernsey cows were studied, and the data on their initial and reëntry butterfat records are listed in table 1. The coefficients of correlation vary from 0.8591 ± 0.0442 to 0.3290 ± 0.1228.   Six other correlation coefficients are above 0.7, six above 0.6, four above 0.5, while only two fall below 0.5. The average correlation coefficient is 0.6605.   The requirements for entry in the Advanced Register increase from two years up to five years of age, and the higher requirement at the reëntry age automatically excluded a certain proportion of records which might have been lower than the initial records made by the same individuals.   Of the 674 animals in this study, 593 could have qualified for reëntry at the ages shown, even though they had produced less fat on the retest.   Of these 593 animals, 43, or 7.25 per cent, made less fat on reëntry test than on initial test.

Data on 33 groups of Jerseys with initial and reëntry butterfat records are shown in table 2.   The correlation coefficients vary from 0.8116 ± 0.0482 to 0.3391 ± 0.1107, with an average of 0.6071.   Six others are over 0.7 and nine over 0.6.   Seven are over 0.5, six over 0.4, and four over 0.3.   Twenty-three are above 0.5 and ten below.   Four hundred seventy-five of the 2419 cows would have been excluded from reëntry by the requirements if the reëntry record had been less than the initial record.   Of the other 1944 animals, 293, or 15 per cent, produced less on retest than on the initial test.

Sixteen groups of Ayrshire cows with initial and reëntry milk records were studied, and the data are listed in table 3.   The highest coefficient of correlation is 0.6445 ± 0.0861, and the lowest is 0.0826 ± 0.0391.   The average coefficient of correlation is 0.5021.   Three others are over 0.6, five are over 0.5, two are above

TABLE 1

Correlation coefficients of initial and reentry records made by Guernsey cows at various ages

NUMBER OF COWS	INITIAL RECORD AGE	REENTRY RECORD AGE	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	COEFFICIENT OF CORRELATION
	years	*years*	*pounds*	*pounds*		
46	2 to 2½		424±8	76±5	18.4±1.3	+0.7390±0.0452
		3 to 3½	501±10	100±7	20.0±1.4	
106	2 to 2½		448±6	90±4	20.1±0.9	+0.7247±0.0311
		3½ to 4	551±8	122±6	22.1±1.0	
50	2 to 2½		427±9	92±6	21.5±1.5	+0.6953±0.0494
		4 to 4½	541±12	121±8	22.4±1.5	
70	2 to 2½		435±6	77±4	17.7±1.0	+0.6059±0.0510
		4½ to 5	552±9	106±6	19.2±1.1	
36	2 to 2½		418±12	103±8	24.6±2.0	+0.6549±0.0642
		5 to 5½	596±17	152±12	25.5±2.0	
34	2 to 2½		416±10	87±7	20.9±1.7	+0.7152±0.0565
		5½ to 6	574±13	115±9	20.0±1.6	
34	2 to 2½		430±9	81±7	18.8±1.5	+0.6553±0.0660
		6 to 6½	600±14	119±10	19.8±1.6	
43	2½ to 3		448±8	77±6	17.2±1.3	+0.5766±0.0686
		3½ to 4	529±9	85±6	16.1±1.2	
47	2½ to 3		447±9	96±7	21.4±1.5	+0.7936±0.0364
		4 to 4½	544±13	137±10	25.2±1.8	
27	2½ to 3		434±9	73±7	16.8±1.5	+0.5080±0.0962
		4½ to 5	547±14	108±10	19.7±1.8	
23	2½ to 3		455±10	70±7	15.4±1.5	+0.5331±0.1005
		5 to 5½	558±16	117±12	21.0±2.1	
20	3 to 3½		421±11	74±8	17.6±1.9	+0.4955±0.1138
		4 to 4½	550±14	94±10	17.1±1.8	
30	3 to 3½		442±11	88±8	19.9±1.7	+0.6506±0.0710
		4½ to 5	567±10	83±7	14.6±1.3	
18	3 to 3½		422±11	70±8	16.6±1.9	+0.7501±0.0696
		5 to 5½	574±14	89±10	15.5±1.7	
24	3 to 3½		459±11	82±8	17.9±1.7	+0.3290±0.1228
		5½ to 6	565±11	77±7	13.6±1.3	
17	3½ to 4		479±15	89±10	18.6±2.2	+0.5959±0.1056
		4½ to 5	556±17	103±12	18.5±2.1	
19	3½ to 4		443±14	91±10	20.5±2.2	+0.6945±0.0801
		5 to 5½	546±18	117±13	21.4±2.3	
14	4 to 4½		506±19	103±13	20.4±2.6	+0.7872±0.0686
		5½ to 6	580±21	119±15	20.5±2.6	
16	4 to 4½		464±13	76±9	16.4±2.0	+0.8591±0.0442
		6½ to 7	566±20	117±14	20.7±2.5	

Average coefficient of correlation, 0.6605.

TABLE 2

Correlation coefficients of initial and reentry butterfat records made by Jersey cows at various ages

NUMBER OF COWS	INITIAL RECORD AGE	REENTRY RECORD AGE	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	COEFFICIENT OF CORRELATION
	years	*years*	*pounds*	*pounds*		
51	1½ to 2		340±6	60±4	17.6±1.2	+0.6597±0.0533
		2½ to 3	412±8	87±6	21.1±1.4	
127	1½ to 2		357±5	76±3	21.3±0.9	+0.7057±0.0300
		3 to 3½	452±6	100±4	22.1±0.9	
40	1½ to 2		342±7	64±5	18.7±1.4	+0.6221±0.0654
		3½ to 4	480±10	91±7	12.8±1.0	
71	1½ to 2		343±6	71±4	20.7±1.2	+0.4845±0.0612
		4 to 4½	508±8	106±6	20.9±1.2	
240	2 to 2½		384±4	83±3	21.6±0.7	+0.6318±0.0262
		3 to 3½	477±5	116±4	24.3±0.7	
218	2 to 2½		395±4	86±3	21.8±0.7	+0.6940±0.0237
		3½ to 4	494±5	118±4	23.9±0.8	
168	2 to 2½		367±4	68±3	18.5±0.7	+0.5877±0.0341
		4 to 4½	496±6	107±4	21.6±0.8	
152	2 to 2½		391±5	83±3	21.2±0.8	+0.5754±0.0366
		4½ to 5	553±7	124±5	22.4±0.9	
105	2 to 2½		388±6	88±4	22.7±1.0	+0.5478±0.0326
		5 to 5½	535±8	115±5	21.5±1.0	
90	2 to 2½		375±5	77±4	20.5±1.0	+0.5818±0.0470
		5½ to 6	563±10	145±7	25.8±1.3	
116	2½ to 3		395±5	77±3	19.5±0.8	+0.7736±0.0252
		3½ to 4	485±6	96±4	19.7±0.9	
99	2½ to 3		404±6	88±4	21.8±1.0	+0.7105±0.0336
		4 to 4½	523±9	128±6	24.5±1.2	
53	2½ to 3		407±9	96±6	23.6±1.5	+0.6103±0.0581
		4½ to 5	514±11	116±8	22.6±1.5	
60	2½ to 3		399±6	72±4	18.0±1.1	+0.3707±0.0751
		5 to 5½	535±11	123±8	23.0±1.4	
84	3 to 3½		420±6	87±7	20.7±1.1	+0.7190±0.0355
		4 to 4½	503±8	115±6	22.9±1.2	
68	3 to 3½		434±7	86±5	19.8±1.1	+0.6138±0.0509
		4½ to 5	516±9	109±6	21.1±1.2	
56	3 to 3½		419±8	92±6	22.1±1.4	+0.6701±0.0497
		5 to 5½	525±8	88±6	16.8±1.1	
53	3 to 3½		420±8	88±6	21.0±1.4	+0.5881±0.0606
		5½ to 6	518±10	109±7	21.0±1.4	
61	3½ to 4		423±7	82±5	19.4±1.2	+0.4626±0.0679
		4½ to 5	514±9	109±7	21.2±1.3	
57	3½ to 4		446±8	85±5	19.1±1.2	+0.6668±0.0496
		5 to 5½	532±9	102±6	19.2±1.2	
41	3½ to 4		416±8	79±6	19.0±1.4	+0.3886±0.0895
		6 to 6½	495±10	99±7	20.0±1.5	

TABLE 2—*Continued*

NUMBER OF COWS	INITIAL RECORD AGE	REENTRY RECORD AGE	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	COEFFICIENT OF CORRELATION
	years	*years*	*pounds*	*pounds*		
66	4 to 4½		436±7	81±5	18.6±1.1	+0.4767±0.0642
		5 to 5½	488±8	95±6	19.5±1.1	
53	4 to 4½		454±7	74±5	16.3±1.1	+0.4070±0.0773
		5½ to 6	540±9	96±6	17.8±1.2	
39	4½ to 5		449±8	74±6	16.5±1.3	+0.4997±0.0810
		5½ to 6	513±11	103±8	20.1±1.5	
29	4½ to 5		479±12	97±9	20.3±1.8	+0.3391±0.1107
		6 to 6½	567±12	97±9	17.1±1.5	
26	5 to 5½		456±10	77±7	16.9±1.6	+0.5842±0.0871
		6 to 6½	508±12	90±8	17.7±1.7	
35	5 to 5½		477±12	106±9	22.2±1.8	+0.7325±0.0356
		6½ to 7	556±9	140±11	25.2±2.0	
36	5½ to 6		457±9	83±7	18.2±1.4	+0.4092±0.0936
		6½ to 7	509±12	106±8	20.8±1.7	
24	5½ to 6		510±18	131±13	25.7±2.5	+0.7768±0.0546
		7 to 7½	573±17	124±12	21.6±2.1	
34	6 to 6½		494±10	86±7	17.4±1.4	+0.5946±0.0748
		7 to 7½	549±11	93±8	16.9±1.4	
23	6 to 6½		523±16	116±12	22.2±2.2	+0.8116±0.0480
		7½ to 8	549±18	126±13	23.0±2.3	
25	6½ to 7		452±13	97±9	21.5±2.1	+0.6592±0.0763
		7½ to 8	519±14	105±10	20.2±1.9	
19	7 to 7½		489±12	79±9	16.1±1.8	+0.3731±0.1332
		8 to 8½	510±15	95±10	18.6±2.0	

Average coefficient of correlation, 0.6071.

0.4, and four are over 0.2; making nine over and seven below 0.5. The breed requirements excluded 145 cows from reëntry at lower than their initial records, and of the remaining 436 cows, 107, or 24.5 per cent, failed to exceed their initial records when retested. All groups have an average coefficient of correlation of 0.5021.

Of a total of 68 groups of animals for the three breeds, only 2 have correlation coefficients exceeding 0.8; 12 are over 0.7; and 49 are above and 19 below 0.5. The 19 groups below 0.5 include 750 of the total of 3674 cows. Thirty-five of the 68 groups have correlation coefficients less than 0.6. The grand average correlation coefficient is +0.6011.

Table 4 shows the distribution of the groups according to their correlation coefficients.

There is a slight tendency for the correlations to decline as the lapse of time between initial and retest records increases.   How-

TABLE 3

Correlation coefficients of initial and reentry milk records made by Ayrshire cows at various ages

NUMBER OF COWS	INITIAL RECORD AGE	REENTRY RECORD AGE	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	COEFFICIENT OF CORRELATION
	years	*years*	*pounds*	*pounds*		
58	2 to 2½		8189±136	1532±96	18.7±1.2	+0.6015±0.0565
		3 to 3½	9577±178	2010±126	21.0±1.3	
48	2 to 2½		9080±202	2073±143	22.8±1.6	+0.5658±0.0663
		3½ to 4	10441±278	2861±197	27.4±1.9	
32	2 to 2½		8251±213	1789±151	21.7±1.8	+0.2917±0.1090
		4 to 4½	10734±222	1866±157	17.4±1.5	
50	2 to 2½		8499±172	1808±122	21.3±1.4	+0.4808±0.0733
		4½ to 5	11783±255	2676±180	22.7±1.5	
78	2½ to 3		9272±128	1679±91	18.1±1.0	+0.6083±0.0481
		3½ to 4	10172±151	1979±107	19.5±1.1	
56	2½ to 3		9569±174	1928±123	20.1±1.3	+0.5782±0.0600
		4 to 4½	10736±208	2310±147	21.5±1.4	
45	2½ to 3		9015±170	1687±120	18.7±1.3	+0.2503±0.0375
		4½ to 5	11003±183	1820±129	16.5±1.2	
38	2½ to 3		8930±169	1536±119	17.2±1.3	+0.2632±0.0338
		5 to 5½	11700±286	2605±201	22.3±1.7	
30	2½ to 3		8710±162	1320±115	15.2±1.3	+0.0826±0.0391
		6 to 6½	12456±296	2407±209	19.3±1.7	
30	3 to 3½		9167±204	1659±144	18.1±1.6	+0.6345±0.0735
		4 to 4½	10556±235	1912±166	18.1±1.6	
25	3 to 3½		9751±253	1878±179	19.3±1.8	+0.5792±0.0896
		4½ to 5	11151±324	2402±229	21.5±2.1	
20	3 to 3½		8914±212	1405±150	15.8±1.7	+0.2530±0.0543
		5½ to 6	11986±396	2623±280	21.9±2.3	
18	3½ to 4		9549±279	1753±190	18.4±2.1	+0.5746±0.1065
		4½ to 5	10961±321	2016±227	18.4±2.1	
16	3½ to 4		9583±249	1479±176	15.4±1.8	+0.5358±0.1202
		5 to 5½	10379±247	1468±175	14.1±1.7	
16	4 to 4½		9463±242	1433±171	15.1±1.8	+0.4138±0.1397
		5 to 5½	10423±278	1646±196	15.8±1.9	
21	4 to 4½		10024±363	2465±257	24.6±2.6	+0.6445±0.0861
		5½ to 6	11613±442	3002±312	25.9±2.7	

Average coefficient of correlation, 0.5021.

ever, there are not enough groups in all ages to make this tendency very pronounced.

While the correlation coefficients constitute the most significant of the calculated data of this study, it will be seen that there is a markedly greater variability among the reëntry records than among the initial records. In only 18 of the 68 groups the coefficient of variability of the reëntry records exceeded that of the initial records; and only 5 groups had greater standard deviations for the reëntry than for the initial records. This may be in part due to the action of breed entry requirements, as they set only a low limit for entry, which does not offset the greater upward range of productive ability that comes with increasing age. There is no other significance shown in the coefficients of variability, and

TABLE 4

Distribution of correlation coefficients of initial and reentry records of cows of various breeds

CORRELATION COEFFICIENT RANGE	GUERNSEY	JERSEY	AYRSHIRE	TOTAL
+0.8000 to 0.8999	1	1		2
0.7000 to 0.7999	6	6		12
0.6000 to 0.6999	6	9	4	19
0.5000 to 0.5999	4	7	5	16
0.4000 to 0.4999	1	6	2	9
0.3000 to 0.3999	1	4		5
0.2000 to 0.2999			4	4
0.1000 to 0.1999				
0.0000 to 0.0999			1	1

for the entire lot they will average close to 20 per cent, with approximate limits of 15 per cent and 25 per cent.

If the original premise is correct, that the free action of heredity alone would result in correlation coefficients of plus one between initial and reëntry records made by the same cows at corresponding ages, then the fact that only 2 of 68 groups in this study have correlation coefficients above 0.8, only 12 others above 0.7, and that 33, or less than half, are above 0.6, while 19 are under 0.5, would seem to indicate that environment has acted to reduce the value of official records as material for studying inheritance of milk and butterfat production. The average correlation of all groups is 0.6071. It might be added that in 443, or 15 per cent, of a possible 2973 cases, the reëntry record did not exceed the initial record.

SWEETENED CONDENSED MILK

V. RANCIDITY[*][1]

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At the time when we gave evidence of the presence of lipase in milk (1), the statement was made that rancidity in sweetened condensed milk is in all probability due to the action of that enzyme; it reaching the manufactured product through the accidental admixture of unheated milk. Data here presented is in support of that conclusion.

The term "rancidity" is here taken to mean the flavor resembling butyric acid. Recently in the discussion of milk products this seems to be the common understanding of the term; such flavors as tallowy, fishy and metallic being carefully distinguished. The rancid flavor is due to butyric acid and perhaps other low molecular weight fatty acids, which result probably always from the hydrolytic splitting of the milk fat.

Rancidity in condensed milk is a defect fortunately not very common, but most serious when it does occur since the flavor is so penetrating and disagreeable that a rancid batch withdrawn from the trade cannot be used in candy making nor any other way as food.

The flavor once observed is never forgotten. It appears usually not before the product is ten days old. In the early stages the flavor of the milk itself may be satisfactory and only by making it up with some hot beverage is the taste noticeable. However, it rapidly becomes worse and in thirty to sixty days the penetrating odor of butyric acid is noted immediately on opening the can. The product becomes gradually more viscous and finally solid; and the acidity increases.

Samples representing about 20 commercial batches of rancid

condensed milk were obtained for laboratory experimentation. The ages of some of the samples were unknown but most of them were two to four months old. On these samples a large number of analyses were made, both biological and chemical; the following observations only seemed significant: The acidity was always high; the bacterial count usually relatively low; and the peroxidase test in almost every case, positive. Table 1 gives the

TABLE 1

SAMPLE	ACIDITY	PEROXIDASE TEST	BACTERIAL COUNT
1	0.892	Strong	
2	1.035	Strong	<500
3	0.954	Strong	
4	1.143	Weak	500
5	1.260	Strong	31,000
6	1.032	Strong	70,000
7	1.125	Strong	<500
8	1.332	Strong	<500
9	0.567	Strong	11,000
10	0.540	Very strong	3,800
11		Very strong	1,700
12	0.882	None	
13	0.792	None	<500
14	0.530	Weak	250
15	0.600		500
16	0.360	Strong	300
17	0.470		500
18	1.017	Strong	
19	0.520	Strong	
20	0.820	Strong	

results obtained. Acidity was determined by titrating a diluted sample with alkali and calculating results in terms of lactic acid. Bacterial counts were made on agar plates.

At first sight it might appear that the condition of rancidity is similar to that of bacterial thickening which was described in the first paper of this series (2), but this is not the case. The acidity does rise in bacterial thickening but usually not so high as in rancidity; in both cases the product becomes thick and gradually

solid. But millions of bacteria are present in the former case, and the differences in flavor are unmistakable.

THE PRODUCTION OF RANCIDITY EXPERIMENTALLY

The fact that the rancid flavor of butyric acid would most likely be produced through the lipolytic splitting of butter fat, the fact already demonstrated that raw milk normally contains lipase, and the fact that peroxidase is found in most of the commercial samples of rancid condensed milk, strongly point to the possibility that rancidity is due to the accidental incorporation of unheated milk in the batch.

Experiments were carried out in two general ways: (a) manufacture of small batches in an experimental vacuum pan and (b) making various additions to normal condensed milk of factory manufacture. Samples were held in regular factory tin cans, one being opened at each time an inspection was to be made. The fact that each analysis is on a different sample explains in part why some of the analyses are rather divergent, particularly the bacterial counts.

EXPERIMENTAL BATCHES CONTAINING SMALL PERCENTAGES OF RAW MILK

A vacuum pan was used of about 400 pounds capacity. It was equipped so that the usual conditions of factory operation could be exactly duplicated. The hot well was equipped for heating the milk by injecting live steam. Unless otherwise stated the milk was brought to a boil in the hot well and the sugar dissolved in it; immediately, then, drawing of milk into the pan was begun. The temperature of condensation was always between 130 and 140°F.

Experiment 1

Batch A. Whole milk 250 pounds, sugar 46 pounds. After all the milk was in the pan, 5 pounds of unheated milk was drawn in.

Batch B. Carried out in a similar manner. Whole milk 170 pounds, sugar 30 pounds, cold unheated milk 13 pounds.

Batch C. Whole milk 170 pounds, sugar 30 pounds, unheated milk 19 pounds.

Batch D. Whole milk 186 pounds, sugar 31 pounds, no unheated milk.

Complete data on these batches is given in table 2. The samples on which the determinations were made had been kept at room temperature; a few were incubated at 98°F. for observation as to the speed in which the rancid flavor developed.

TABLE 2

	BATCH A	BATCH B	BATCH C	BATCH D
Per cent of unheated milk....................	2	7	10	0
Per cent of total solids in finished batch:.....	74.00	74.56	75.33	75.45
Peroxidase test................................	Fair	Strong	Very strong	None
Acidity (per cent):				
Beginning......................	0.351	0.342	0.360	0.342
End of 10 days................	0.396	0.342	0.432	
End of 20 days................	0.414	0.405	0.477	0.342
End of 30 days................	0.450	0.513	0.486	0.315
End of 60 days................	0.490	0.570	0.540	0.360
End of 4 months..............	0.510	0.560	0.640	0.300
Bacterial counts:				
End of 5 days................	4000	7500	63,000	4500
End of 17 days................	2400	3300	22,000	
End of 27 days................	4500	3400	9,600	
End of 37 days................	2500	2500	6,600	11,200
End of 47 days................	1100	1170	2,300	
End of 3 months..............				3200
End of 5 months..............	850	950	450	
Time of appearance of the rancid flavor:				
Incubated samples............	10 days	10 days	10 days	Not in
Room temperature samples....	30 days	30 days	10 days	6 months

Excepting the samples from the check batch (*D*) a gradual increase in acidity was noted in all cases and a flavor developed exactly like that of the rancid commercial samples previously described. Also the peroxidase test was always given. The general decrease in bacterial counts found here is not different from the behavior of normal condensed milk. Apparently those bacteria which contaminate the product at the time of manufac-

ture find it an unfavorable medium and all die out except a few species that are resistant to the high concentration of sugar.

The evidence presented to this point together with that to follow is sufficient to warrant the conclusion that rancidity in sweetened condensed milk results from the action of the lipase of raw milk on the butter fat; the liberation of free fatty acids, at least in part, causes the increase in acidity; those of lower molecular weight being the cause of the disagreeable odor.

These results show that the increase in rancidity can be meas-

TABLE 3

| | RAW MILK ADDED | | | | | | | |
| | 0 per cent | | 1 per cent | | 2 per cent | | 4 per cent | |
	I*	R†	I	R	I	R	I	R
Acidity (per cent):								
Beginning.............	0.28	0.28	0.28	0.28	0.28	0.28	0.26	0.26
End of 30 days........		0.28	0.43	0.36	0.48	0.42	0.56	0.49
End of 60 days........	0.31		0.43		0.48		0.58	
End of 3 months......	0.33			0.43		0.51		0.81
End of 6 months......				0.62		0.52		0.82
Rancidity:								
End of 30 days........	0	0	+		+		++	
End of 60 days........	0	0	+	0	++	+	+++	+
End of 3 months......	0	0		++		++		+++

* Incubated = I.

† Room temperature = R.

+ indicates moderate rancidity; +++ strong; 0 none.   Blank spaces indicate that no observations were made.

ured at least in a rough quantitative way by the increase in acidity.   For this reason the acidity of samples is given also in the remaining experiments.

ADDITIONS OF RAW MILK TO NORMAL CONDENSED MILK

Experiment 2

A quantity of condensed milk was obtained from a factory batch shortly after manufacture.   To portions of this were added 1 per cent, 2 per cent, and 4 per cent of raw milk.   One portion was

kept without any addition as a check. Samples were held both at incubator and room temperatures.

Table 3 gives the changes in acidity and the time of appearance of the rancid flavor. These results show that 1 per cent of raw milk is sufficient to cause rancidity at incubator temperature in thirty days and at room temperature in three months. The larger additions caused a more rapid development of the flavor. The acidity figures are in line with the inspections for flavor, very marked increases taking place except in the check. The very slight increase noted in the check sample is similar to that usually observed when normal condensed milk becomes old particularly when stored at higher than normal temperatures. As these results show, however, the acid increase in rancid samples is so much more rapid than in the check sample that there is no danger of confusion.

THE AMOUNT OF UNHEATED MILK NECESSARY TO PRODUCE RANCIDITY

Experiment 3

To different portions of normal condensed milk was added 0.1 0.5, 0.75, and 2 per cent of raw milk. In table 4 will be found the results of titration for acidity and of inspection for flavor.

Five-tenths per cent raw milk did not produce rancidity in four months, neither was lipolytic activity indicated by titration. However, in samples to which 0.75 per cent raw milk had been added, rancidity occurred of such degree as should prove objectionable to the trade. Of course, 2 per cent raw milk acted much more rapidly. The smallest amount found necessary to produce rancidity (0.75 per cent) calculated on the basis of the original whole milk in the sample would be equal to 0.3 per cent, considering the condensation to be $2\frac{1}{2}$ times. This shows even more how powerful is the effect of a little unheated milk. On a batch of 20,000 pounds of milk to produce rancidity would be required only 60 pounds of raw milk—much less than one can!

TEMPERATURE REQUIRED TO DESTROY LIPOLYTIC ACTIVITY

Experiment 4

To different portions of normal condensed milk were added 3 per cent portions of whole milk which had been exposed to different heat treatments as follows:

Series A. Milk was brought to the temperature indicated and cooled at once—(1) 170°, (2) 160°, (3) 150°, (4) 140°F.

Series B. Milk was brought to the temperature indicated,

TABLE 4

| | PER CENT RAW MILK | | | | | | | |
| | 0.10 | | 0.50 | | 0.75 | | 2.00 | |
	I*	R†	I	R	I	R	I	R
Acidity (per cent):								
Beginning.............	0.320	0.320	0.320	0.320	0.320	0.320	0.320	0.320
End of 20 days........	0.342		0.324		0.378		0.414	
End of 35 days........	0.351		0.423		0.495		0.522	
End of 75 days........	0.370		0.390				0.510	
End of 4 months......		0.310		0.340		0.380		0.460
End of 5 months......		0.310		0.360		0.430		0.490
End of 9 months......		0.360				0.400		0.550
Rancidity:								
End of 20 days........	0		0		0		+	
End of 35 days........	0		0		?		+	
End of 75 days........	0		0		+		++	
End of 4 months.....		0		0		++		++

* Incubated = I.
† Room temperature = R.

held for fifteen minutes, then cooled—(1) 170°, (2) 160°, (3) 150°, (4), 140°, (5) 130°, (6) 120°F.

Sample C. As check, no heat treatment.

Of series A, no. 4 only became rancid. The increase in acidity in eight months at room temperature was from 0.30 to 0.43 per cent; under the same conditions the check sample increased to 0.59, which indicates that there was some destruction of lipase even at that temperature.

Among those of series B, nos. 1, 2, 3, and 4 did not become ran-

cid. Number 5 was rancid in four months, and 6 in two months.

The results indicate that heating for an instant at 150°, or, for fifteen minutes at 140° is sufficient to destroy lipase activity. It may be mentioned that on one occasion an entire batch was prepared by heating the milk at 145°—thirty minutes when no randicity developed.

This data gives some idea of the heat exposure necessary for the destruction of lipase. More exact figures were not obtained because it was believed that they would not prove to be useful, since conditions in the factory cannot well be duplicated in the laboratory. The next experiment shows how much, indeed, the conditions may influence the resistance of this enzyme to heat.

EFFECT OF THE PRESENCE OF SUGAR ON THE TEMPERATURE REQUIRED TO DESTROY LIPOLYTIC ACTIVITY

On several occasions samples of condensed milk containing some raw milk were heated to 180° for thirty minutes for the purpose of killing the lipase, but not always with success. This led to some experiments to learn whether or not the sugar had a protective influence.

Experiment 5

Two portions of raw milk containing 35 per cent cane sugar were heated and cooled at once: (1) to 170°, (2) to 185°F. These were added to condensed milk in 3 per cent proportions. Samples of series 1 became rancid in thirty days, those of series 2 not in six months.

Experiment 6

To a quantity of condensed was added 6 per cent of raw milk; this was divided into three portions: (1) no treatment, (2) heated to 180° for ten minutes, (3) heated to 200° for ten minutes. Samples of 1 and 2 became rancid in thirty days, those of series 3 not in six months; at the end of four months series 1 had increased in acidity from 0.27 to 0.64 per cent and series 2 from 0.28 to 0.54, indicating practically no lipase destruction at 180° ten minutes.

These results may be compared with those obtained in experiment 4, where it was found that by heating raw milk alone to 150° for an instant was sufficient to destroy the lipolytic power. From this it is evident that in the presence of sugar (or perhaps where less moisture is present), there is a protection of the enzyme from destruction by heat.

LIPOLYTIC POWER OF OLD MILK COMPARED WITH FRESH. THE RELATION OF BACTERIA TO RANCIDITY

Experiment 7

To two portions of condensed milk fresh raw milk was added in the proportions of 1.5 and 2.5 per cent. The remainder of the

TABLE 5

	CONDITION OF RAW MILK			
	Fresh		3 days old	
	Per cent added		Per cent added	
	1½	2½	1½	2½
Acidity (per cent):				
Beginning..........................	0.28	0.28	0.29	0.29
End of 30 days...................	0.38	0.46	0.31	0.41
End of 60 days...................	0.51	0.57	0.43	0.54
Rancidity:				
End of 30 days...................	0	++	0	0
End of 60 days...................	+	++	0	++

sample of raw milk was allowed to stand at room temperature for three days when it had curdled. Then another series of samples was prepared exactly like the first.

Table 5 gives the results of titrations for acidity and inspections for rancidity. It is shown there that the power of old milk to produce rancidity is no greater than that of fresh milk; in fact, it seems to be even less.

Throughout these experiments no direct evidence has been presented to support the contention that it is a lipase natural in milk which causes rancidity and that it is not due to a strain of organisms always present in raw milk. In all such cases as this,

it is extremely difficult to present any evidence other than indirect; and there is no question but that every one of the experiments here recorded point to the conclusion that it is an enzymic effect; a lipase natural in milk and not one produced by organisms. The experiment just described, does, indeed, support that conclusion: If the rancidity-producing power of raw milk were due to bacteria always present therein, the effect of aging should be to greatly increase that power; but the opposite was found to be the real state of affairs.   Of course, there can be no increase in the amount of natural lipase, present in milk, the only quantitative change that can take place would be that its activity might be reduced in time.

On the assumption that in some special cases rancidity might possibly be produced through bacterial action, a large number of experiments were carried out.

A sample of raw milk was plated out under both aerobic and anaerobic conditions.   Cultures of all organisms were grown in sterile milk.   A commercial sample of rancid condensed milk was plated out and handled similarly.   All cultures were added to condensed milk with the result that rancidity was produced in no case.   The sample of raw milk, however, from which the first series of cultures was made produced rancidity when tested by the methods already described.

Also batches were prepared in the experimental vacuum pan using milk which had first been sterilized then inoculated with bacteria from the above-mentioned and other sources and a large number of molds.   In no instance did rancidity develop.

ATTEMPT TO FIND OTHER CAUSES OF RANCIDITY

By running experimental batches and through additions to normal condensed milk the following were also eliminated as causes of rancidity: Applying excessive heat to milk or cream before running the batch, condensing at extremely high pan temperatures, use of sour milk neutralized with sodium bicarbonate, freezing the milk used in the batch, addition of various oxidation and hydrolytic products of fat, addition of salts of iron, copper, tin, zinc and hypochlorous acid.

Batches have been known to become rancid in which the content of cane sugar was so high that it crystallized out, also rancid batches were prepared in which the total solids was more than 77 per cent. But batches of low sugar and low solids content became rancid just as readily.

In fact 70 batches and numerous samples prepared in various ways are not described in this report. In not a single instance was there any indication that there is another cause of the typical butyric acid-like rancid flavor than through the action of a lipolytic enzyme which was found to be present in all the specimens of raw milk on which there was occasion to experiment.

RANCIDITY PRODUCTION BY THE ACTION OF PANCREATIC LIPASE

A suspension in water of commercial pancreatin was added to normal condensed milk in such a proportion that about 0.2 per cent of the pancreatin powder was present. The acidity of the mixture at the beginning was 0.30 per cent; at the end of forty days at room temperature 1.43 per cent, and at six months 2.73 per cent. Samples became gradually thicker and finally solid as was always found in rancid samples, and the odor was exactly that of rancid condensed milk as it is invariably found.

SUMMARY

1. Rancidity here refers to that flavor resembling butyric acid.

2. Commercial samples of rancid condensed milk were found to run relatively high in acidity and in most cases to give the peroxidase test. The viscosity was higher than in normal condensed milk; in some cases the product was solid.

3. Experimental batches of condensed milk prepared under usual conditions except that a little raw milk was allowed to enter the pan while the batch was being condensed invariably became rancid. The defect was more marked, the more raw milk present.

4. The addition of unheated milk to normal condensed milk always produced rancidity, as little as 0.75 per cent being effective.

This is equivalent to 0.3 per cent of the amount of whole milk from which the batch was prepared.

5. Heating whole milk to 140°F. for fifteen minutes, or 150° for an instant, was found sufficient to destroy lipolytic power. However, when sugar was present, or, where attempts were made to destroy lipase in condensed milk, a considerably higher temperature was required.

6. Milk three days old had no more power to produce rancidity than fresh milk. This is presented as a point of evidence that this power is not due to any bacteria that might be present.

In further support of this contention an experiment was carried out in which cultures of all organisms isolated from raw milk failed to produce rancidity when incorporated in normal condensed milk, though the raw milk itself did have the power to do so. Cultures of organisms isolated from rancid condensed milk also failed to produce rancidity.

Sterile whole milk inoculated with these and other cultures, then condensed down in the usual way did not produce rancid batches.

7. Various metallic salts and other chemicals and various mechanical treatments of milk did not cause rancidity. Its production was independent of solids or sugar content.

8. Pancreatic lipase added to condensed milk was shown to produce the rancid flavor, increase the acidity and viscosity exactly as did raw milk.

THEORETICAL CONCLUSIONS

1. The cause of rancidity in condensed milk is the accidental incorporation of raw milk in the batch. The activity of the raw milk is due to the presence naturally of a lipolytic enzyme, which splits the milk fat yielding among other fatty acids those of low molecular weight such as butyric, to which the rancid flavor is due.

2. Data given here may be considered further proof of the presence of lipase in milk in addition to that already presented by us (1).

3. The importance of considering milk products with this point

in mind is again emphasized.   Rancidity (butyric acid-like flavor) in cream and milk, butter and cheese may well be attributed to this enzyme.

4. It is common knowledge that the best milk products can be manufactured only from good fresh milk.   Bacterial action may not be the only factor entering here.   When the milk is old, lipase and perhaps other hydrolytic enzymes have time to produce small amounts of substances which may later lower the quality of the manufactured product.   The work of Holm and Greenbank (3) at Washington has shown that the free fatty acids increase the oxidizability and consequent tallowiness of fats.   The most likely source of fatty acids is from the lipolytic splitting of fat.

PRACTICAL CONCLUSIONS

1. The contamination causing rancidity is most likely to take place through raw or improperly heated milk being accidentally drawn into the batch at the time of condensation.

2. The great danger of rancidity is shown by the fact that only 0.3 per cent of unheated milk in a batch may cause this defect.

3. The temperature under which a vacuum pan ordinarily operates (130 to 140°F.) is not sufficient to destroy the rancidity-producing power of raw milk.   Hot well temperatures used in common practice (180°–boiling) are sufficient; though 180°F. is a dangerous temperature when much sugar or condensed milk has been added to the hot well.

4. Two variations from good factory operation that might make rancidity possible are: (a) Leaky intake valves through which a small amount of milk from an unheated hot well might be drawn into a pan under operation, and (b) carelessness in heating the hot well so that all the milk does not reach the temperature necessary to destroy the enzyme.   The latter possibility would include the dangerous practice of pouring a few cans of cream or milk into a hot well which at the same time is being drawn into the pan.

REFERENCES

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JOHAN D. FREDERIKSEN

News has just been received of the death of Mr. J. D. Frederiksen at St. Petersburg, Fla., on February 19, 1926. Mr. Frederiksen was for many years vice-president and general manager of Chr. Hansen's Laboratory, Little Falls, N. Y., having retired from active business in the fall of 1924. He was born at Fuglsang, Lolland, Denmark, August 18, 1846. His father was an expert farmer who at one time conducted eight large estates, two of which he owned. His mother was the daughter of a minister named Hansen.

He attended school in Soro where he lived in the home of Professor Johnstrup, later graduating from the agricultural college in Copenhagen in 1866. A few years later, he and his brother Christian attempted to introduce sugar beet raising in Denmark. Production began in 1872. Then came the "Black Friday" on the New York Stock Exchange precipitated by Jay Gould. The effect was disastrous even in Denmark, and ruined the two brothers financially. Later, however, Mr. Frederiksen was decorated for his activity in this work with the Order-Dannebrog by the Danish King.

He made a new start in coming to New York as a representative of the Chr. Hansen Laboratory on November 9, 1877. Here he developed the laboratory at Little Falls, and later at Toronto and Milwaukee. His knowledge of milk led him to develop "Junket," his outstanding achievement, well known to every housewife. His business activities made him known and respected throughout the dairy industry of America, and his frequent visits to the parent laboratory in Copenhagen kept him in touch with his homeland.

Books, music, pictures, family, friends, woods and lakes, politics, fishing, business—he knew and loved them all through most of his eighty busy years. The world is poorer when such a man dies.—*Condensed by R. S. Breed from an account published in the Utica (N. Y.) Press, February 21, 1926.*

THE EFFECT OF FAT IN THE RATION UPON THE PERCENTAGE FAT CONTENT OF THE MILK*

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An attempt to increase the percentage butterfat content of cow's milk has been the object of a large number of experiments. This subject engaged the efforts of several investigators during the early years of the agricultural experiment stations in this country, and has received no little attention also on the part of research workers in Germany, The British Isles, France, Denmark and India. An adequate review of the extensive literature on this subject is precluded by lack of space.

Within recent years, the subject has attracted attention in connection with the making of high production records under the supervision of the Advanced Registry Departments of the dairy cattle breed associations.

The close supervision given these records is intended to assure the accuracy of the record and the prevention of fraud through changes of the feed or otherwise, during the period of actual inspection by the supervisor or just prior to this period. A number of contributions to the literature on this subject have appeared in this journal (1).

There are several ways in which the percentage of fat in the milk may be materially influenced; e.g., (a) by having the cows excessively fat at the time of calving (2). Both percentage

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fat content and yield of butterfat are increased above normal. This is considered a legitimate practice and is commonly used. (b) By physiological underfeeding, that is shortly after calving, reducing the feed sharply below the requirements (3). This practice, however, usually reduces milk and may reduce the total fat production and is, therefore, little favored. (c) By feeding excessive amounts of certain high protein concentrates, such as cottonseed meal, these being added to the usual ration or substituted for part of it (4), (5). The effects on the composition of the milk induced by such changes in the feed are evidently inconstant and vary greatly with different individuals. Armsby maintains that "both experiments with pure proteins and those in which an increase in the protein content of the rations has been secured by the use of protein-rich feeds, seem to indicate clearly a stimulating influence of excess protein on milk production, although in a majority of cases the effect was not very large." (d) By increasing the fat content of the ration by the addition or substitution of fat or feeds high in fat. The literature dealing with investigations of this sort is extensive. One of the more recent experiments was reported by Woodward (5) in this journal.

In Woodward's experiments, high protein concentrates were substituted for one-fourth or three-fourths of the "ordinary grain," thus presumably greatly increasing the daily intake of protein. Substitution of the smaller amounts was without apparent influence on the test of the milk, but when linseed oil meal and cottonseed meal were substituted for three-fourths of the "ordinary grain" there was in most cases, an effect upon the test for a short time at least. Gluten feed seemed to be without effect. Calculation of the fat yields by means of data on milk yields, kindly supplied to the authors by Mr. Woodward, shows that substituting cottonseed meal for three-fourths of the grain in one period caused an increase of 15 per cent in fat yield, while in the case of linseed oil meal used in the same way, there were increases of from 1 to 10 per cent and decreases of the same magnitude. Usually the substitution of linseed oil meal for three-fourths of the grain mixture caused an increase in the

test of the milk, but in at least two such cases such a change depressed the fat yields 8 and 10 per cent, respectively. The addition of linseed oil to the "ordinary grain" of three cows caused an increase in the test of the milk of about 9 per cent and a decreased yield of fat of more than 5 per cent.

While the percentage of fat in the milk of a cow during a two-day test has more weight than the yield of fat, since the average percentage of fat for the two days is used as the average percentage for the month, a decreased fat yield caused by a depressed milk flow is an undesirable accompaniment. A marked lowering of the milk yield below that of the days preceding or following the test period at once raises a question regarding the correctness of the milk weights as reported by the owner for the balance of the month. In such a case also, changes in the feed or "jockeying" of the cow may be suspected.

In executing the experiments reported herewith, efforts were made to maintain the levels of protein intake at as nearly the exact requirements of the individual animals as possible, and to prevent decreases in the daily milk yields even when extreme changes in the rations were made.

OBJECT OF INVESTIGATION

The primary object of the investigation reported herewith was to study the effect of rations high in oil content upon the composition of the milk produced, especial attention being focused upon the percentage butterfat content. A secondary object in the later experiments, was to establish, if possible, the factor responsible for the changes in the percentage butterfat content of the milk which were noted in the second series of experiments.

GENERAL PLAN OF THE INVESTIGATION

The general plan of feeding the experimental animals (cows) was to feed rations high in oil content and low in oil content during alternate periods, samples of milk for analysis being taken at each milking. In Series II, a concentrate mixture

moderately high in fat content which was used in feeding the general herd constituted the check or control rations, while in the other series, a concentrate mixture formulated as being especially low in fat content was used as a control. The amounts of the feeds supplied were as closely as possible in accord with the estimated requirements of the animals. The experimental periods of Series I and II varied in length from two days to five weeks, while in Series III and IV they were seven days in length.

In Series II determinations of the percentage of fat in the milk were made by the Roese-Gottlieb method, comparison being made with the Babcock method. Determinations of total solids were made in accordance with the methods of the Association of Official Agricultural Chemists. In the other series, the Babcock method for determinations of butterfat was used. Mercury bichloride tablets were used as a preservative agent in Series I, in which butterfat tests were made weekly, while in the other series formalin was the preservative agent used whenever it was necessary. In Series III and IV, a preservative was seldom used because tests were made of the individual samples of each milking, usually within twenty-four hours.

CHARACTER OF RATIONS AND ROUTINE OF THE EXPERIMENTS

The roughage part of the ration during Series I and II consisted of corn silage and alfalfa hay, fed in proportions and amounts intended to meet the maintenance requirements of the cows exactly. In Series III and IV part of the silage and hay was replaced by dried beet pulp which was soaked for ten to twelve hours before feeding. The use of roughage in definite proportions to supply maintenance needs made it possible to feed the concentrates quite closely in proportion to milk production.

Refused feeds were weighed back and the amounts recorded. In a few cases in which cows went off feed, it was necessary to supply temporarily feeds other than the experimental ration, but such changes were also recorded. During Series I, the

experimental rations were interchanged gradually, while in the other series the changes in rations were made abruptly.

In making calculations of the amounts of digestible nutrients in feeds, the average analyses as given by Henry and Morrison (6) were used. The requirements for maintenance and milk production were estimated from an average of values given in the Morrison feeding standard (6).

Series I

Cows 668 and 671 were Holstein-Guernsey crossbred animals which were fed during 1921–1922 on "high oil" and "low oil" rations for four-week periods (period III, five weeks) each period being preceded by a one-week transition period in which the rations were gradually interchanged.

The "low oil" concentrate mixture for periods I and II consisted of 100 pounds of wheat bran and 200 pounds dried beet pulp, while for periods III and IV the proportions of the ingredients were reversed. The "high oil" mixture during periods I and II was composed of high oil corn, grown in plant breeding experiments, and containing about 9.9 per cent ether extract and sunflower seed in equal parts by weight. In periods III and IV, the mixture was made up of 200 pounds of high oil corn, 200 pounds sunflower seed and 300 pounds soybean seed. The feeds were mixed and then ground.

Series II

The check concentrate mixture used in Series II, which was carried out during July and August, 1923, consisted of 200 pounds ground corn, 200 pounds ground oats, 200 pounds wheat bran and 100 pounds linseed oil meal. The "high oil" mixture for this series consisted of 250 pounds "high oil" corn, 100 pounds sunflower seed and 150 pounds soybeans. The mixture was ground. Silage made from "high oil" corn containing about 2 per cent ether extract, was substituted for normal silage during the high oil periods. The animals employed were grade Holstein cows.

Series III

In both Series I and Series II, the concentrates comprising the low oil and high oil mixtures came from different plant sources. It was planned that in Series III and IV, which were carried out during 1924 and 1925, respectively, that the concentrate mixtures being compared should be made up of the same kind of feeds and in as nearly the same proportions as possible, with the exception that the high oil mixtures should contain feeds consisting of ground whole seeds, whereas the low oil mixtures should contain feeds resulting from the extraction of the oil from the same kinds of seeds. In this way soybeans were compared with soybean oil meal, peanuts with peanut oil meal, and flaxseed with linseed oil meal. The concentrate mixtures were so planned for Series III that the percentage of digestible protein remained constant throughout the series, regardless of the content of total digestible nutrients and digestible fat. The mixtures were fed at levels intended to meet the estimated requirements as closely as possible. This was accomplished with a considerable measure of success.

The roughage supplied during Series III was fed at a level intended to meet the maintenance requirements and consisted of 20 pounds of corn silage and 8 pounds alfalfa hay daily per 1000 pounds live weight.

The animals used in Series III and IV were purebred dairy cows, with the exception of cows 6 and 25, which were grades. Several of the cows were employed in both series of experiments.

Precautions were taken in Series III and IV of these experiments to insure a level of protein and total digestible nutrient intake in accord with the animals needs, and to avoid so far as possible, changes in the character of the nutrients supplied. In many previous experiments of this nature, one factor, which may have an important bearing upon the interpretation of the causes of observed changes in the composition of milk, was not controlled. This factor was the energy content of the rations. An oil or feed high in oil was usually added to a basal ration, thus making the high oil ration much higher in energy value

than the basal ration used as a check. It is believed that unless experiments can be carried out in which a comparison is made of rations having iso-dynamic values, and fed at levels which exactly meet the needs of the animals, it cannot be definitely proved that differences in the oil content of rations cause differences in the composition of milk produced. In discussing the specific effects of feeds, Armsby (4) points out that:

The effects of such feeds as palmnut meal, cocoa meal and cottonseed meal, for example, are reported by different experimenters as favorable, unfavorable or indifferent.

Defective planning of experiments is doubtless responsible for much of this confusion. In many instances the experimenters have simply added the feed to be tested to a light basal ration. . . . . Others, while substituting one feeding stuff for another, have failed to show that the total amount of digestible matter supplied was unchanged. In some extensive investigations, for instance, oil meals and similar feeds have been interchanged in amounts supplying equal quantities of protein without regard to the other ingredients. Under such conditions concordant results could not be expected and one can but agree with Lemmermann and Linkh that the evidence is inconclusive. . . . .

In Series III and IV, therefore, a special effort was made to balance the rations and to adjust frequently the amounts of concentrates fed so that the requirements would be met closely.

Series IV

The plan followed in Series IV was somewhat similar to that of Series III. Each experiment consisted of five periods, each 7 days in length. During periods I, III and V the oil meal concentrate mixture was fed, while during period II, a concentrate mixture in which the ground whole seeds replaced the oil meal was fed.

It was planned to make the oil content of the oil meal rations and whole seeds rations differ as much as possible while still balancing each mixture to meet the requirements of milk production exactly. This difference was naturally greater when seeds rich in oil, such as peanuts, were fed in comparison with

peanut oil meal, and less in the experiments in which seeds only moderately rich in oil, such as soybeans, were fed. During period IV an amount of oil equivalent to the difference in digestible fat content of the oil meal rations fed in periods I and III, and that of the seeds ration fed in period II, was combined with the oil meal concentrate mixture. In making the combination, the starch was omitted or greatly reduced, and in some cases the quantity of beet pulp was reduced also, in order to keep the energy value of the ration as measured by its content of total digestible nutrients at the same level as during the three previous periods. In making the calculations it was assumed that the oil added was entirely digestible.

<div align="center">DISCUSSION OF RESULTS</div>

Series I

The high oil rations seemed to exert very little, if any, effect upon milk yield (table 1). The amounts of total digestible nturients consumed were practically the same during the high oil and low oil periods (figs. 2 and 3).

The percentage butterfat content of the milk was apparently increased somewhat at the beginning of the high oil feeding, but as the periods advanced, the percentage declined. The average test of the milk for the combined yields of the high oil periods was no higher than for the low oil periods (table 1). The procedure followed in determining the butterfat content of the milk (7-day composites) was evidently not sufficiently detailed for following marked changes in the test, if there were such.

Series II

Samples of milk for analysis were taken beginning at the milking following the one at which the change in feed was made.

The percentage of fat and fat yield for the group in period II, during which the high oil ration was fed, were about 8 per cent greater than the averages of periods I and III, during which the herd ration was fed (table 2). The content of total solids showed

the same trend as the fat content, though the increase was not so marked. The percentage of fat in period IV, another high oil period, was also slightly higher than in the periods preceding and following it. Period IV, however, was 18 days in length, and the effect of the high oil feeding upon the test is better seen by examining the productions during different portions of the period, as is done in table 3. Butterfat was determined in the milk of each milking during the first two days of the period and

TABLE 1

Summary of production of cows, Series I

PERIOD*	cow 668				cow 671			
	Ration	Milk	Fat	Per cent fat	Ration	Milk	Fat	Per cent fat
		pounds	*pounds*			*pounds*	*pounds*	
Preliminary....	Herd	631.6	32.3	5.1	Herd	814.7	34.0	4.2
I.............	High oil	743.7	34.3	4.6	Low oil	801.4	35.9	4.5
II.............	Low oil	690.8	31.0	4.5	High oil	838.8	32.2	3.8
III.............	High oil..	663.5	31.2	4.7	Low oil	796.5	31.2	3.9
IV.............	Low oil	578.9	24.7	4.3	High oil	750.5	28.5	3.8
Subsequent....	Herd	672.9	30.3	4.5	Herd	865.5	31.2	3.6

	MILK	FAT	PER CENT FAT
	pounds	*pounds*	
Total production of both cows during high oil periods.......................	2,996.5	126.2	4.21
Total production of both cows during low oil periods............................	2,867.6	122.8	4.28

* Periods were 4 weeks in length, except period II, which was 5 weeks. For comparison, period II has been corrected to a basis of 4 weeks.

in composite samples representing the other portions of the period.

The average test for the first two days of period IV was nearly 16 per cent higher than the average for the whole period, and 18 per cent higher than the three periods of herd ration feeding. The next three days' test showed a sharp drop. The high oil feeds seemed to have lost their effect upon the test by the close of the fifth day.

TABLE 2

Summary of production of cows, Series II, 1923

PERIOD	RATION	COW	TOTAL SOLIDS		MILK	FAT	TEST
			pounds	*per cent*	*pounds*	*pounds*	
I (10 days)	Herd	571	39.19	12.29	318.9	11.07	3.47
		574	56.43	12.07	467.5	15.95	3.41
		575	41.61	11.55	360.0	11.19	3.11
		576	43.42	11.40	380.6	11.35	2.98
Total..........................			180.65		1,527.0	49.56	
Average per day..................			18.07	11.83	152.7	4.96	3.25
II (3 days)	High oil	571	11.25	12.18	92.4	3.23	3.50
		574	16.18	12.16	133.1	4.86	3.65
		575	11.81	11.89	99.3	3.39	3.41
		576	12.13	11.65	104.1	3.45	3.32
Total..........................			51.37		428.9	14.93	
Average per day.................			17.12	11.98	143.0	4.98	3.48
III (12 days)	Herd	571	43.55	11.95	364.5	11.81	3.24
		574	61.23	11.89	515.0	17.22	3.34
		575	44.25	11.43	387.1	11.97	3.09
		576	47.41	11.45	413.9	12.53	3.03
Total..........................			196.44		1,680.5	53.53	
Average per day.................			16.37	11.69	140.1	4.46	3.19
IV (18 days)	High oil	571	62.03	12.36	501.7	17.50	3.49
		574	72.73	11.91	610.6	21.12	3.46
		575	61.70	11.72	526.3	17.06	3.24
		576	70.06	11.65	601.0	17.93	2.98
Total..........................			266.52		2,239.6	73.61	
Average per day..................			14.81	11.90	124.4	4.09	3.29
V. (21 days)*..	Herd	571			551.7	18.12	3.29
		574			722.4	24.28	3.36
		575			627.3	19.44	3.10
		576			658.7	20.40	3.10
Total..........................					2,560.1	82.24	
Average per day.................					121.9	3.92	3.21

* Percentage of fat determined by Babcock method during period V.

TABLE 3
Detailed production of Period IV, Series II, 1923

COW	MILK	SOLIDS		FAT	TEST
First 2 days					
	pounds	*pounds*	*per cent*	*pounds*	
571	59.0	7.26	12.30	2.100	3.56
574	57.9	7.73	13.35	2.876	4.97
575	60.2	7.02	11.66	2.066	3.43
576	68.4	8.08	11.81	2.3505	3.37
Total...........	245.5	30.09		9.347	
Daily average.....	122.8	15.05	12.26	4.674	3.81
Days 3, 4, 5					
571	86.1	10.87	12.62	3.168	3.68
574	91.6	10.91	11.91	3.207	3.50
575	86.7	10.19	11.75	2.905	3.35
576	102.6	11.86	11.56	3.016	2.94
Total...........	367.0	43.83		12.296	
Daily average.....	122.3	14.61	11.94	4.099	3.35
Days 6, 7, 8					
571	88.1	10.73	12.18	2.969	3.37
574	107.0	12.58	11.76	3.306	3.09
575	88.1	10.43	11.84	2.908	3.30
576	102.4	11.81	11.53	3.021	2.95
Total...........	385.6	45.55		12.204	
Daily average.....	128.5	15.18	11.81	4.068	3.17
Days 9, 10, 11					
571	86.7	10.72	12.36	3.009	3.47
574	109.9	12.84	11.68	3.440	3.13
575	88.0	10.40	11.82	2.834	3.22
576	98.4	11.65	11.84	2.943	2.99
Total...........	383.0	45.61		12.226	
Daily average.....	127.7	15.20	11.91	4.075	3.19
Days 12 to 18					
571	181.8	22.45	12.35	6.253	3.44
574	244.2	28.67	11.74	8.293	3.40
575	203.3	23.66	11.14	6.342	3.12
576	229.2	26.66	11.63	6.646	2.90
Total...........	858.5	101.44		27.534	
Daily average.....	122.6	14.49	11.82	3.933	3.21

The differences in oil content of the rations being compared were relatively small. The calculated daily amounts of digestible fat consumed by the four cows in the first four periods were 3.8, 6.1, 3.7 and 5.7 pounds, respectively. In spite of the small differences, there was a distinct effect of the rations upon the test of the milk. The amounts of digestible protein and total digestible nutrients consumed met the estimated requirements of these substances closely (fig. 4).

A comparison of the Babcock and Roese-Gottlieb methods of determining the percentage butterfat content of milk was made during Series II. It was found that the Babcock method gave results slightly higher (0.04 to 0.13 per cent fat for the different cows) than the Roese-Gottlieb method (table 4).

Series III

This series comprised three feeding trials of three weeks each, covering a total time of about ten weeks. Period I, during which the soybean oil meal ration was fed, was extended to 13 days in order that the cows might become better accustomed to the feed, and also on account of the recent freshening of some of them. The other periods were seven days in length and the records of milk production coincide with the feeding periods.

The number of days the cows had been in milk at the beginning of Series III was as follows: Cow 262, 2 days; cow 270, 49 days; cow 300, 7 days; cow 303, 5 days; cow 316, 309 days; cow 323, 13 days. Cows 6 and 25 had been in milk 87 days and 102 days, respectively, at the beginning of the feeding trials reported for them under experiment B.

The increases in test obtained by replacing soybean oil meal in the concentrate mixture with soybeans were not large, and in one case, that of cow 262, the results were negative (table 5). The results obtained with cow 262 are not conclusive, however, because she continued to increase in milk production throughout both experiments A and B and failed to consume enough nutrients to meet her requirements. The recent freshening of several of the cows seemed to be a factor of minor importance in determining the extent to which the ration affected the test,

TABLE 4

Comparison of Babcock and Roese-Gottlieb methods for determination of butterfat in milk

SAMPLE NUMBER	RATION	COW 571		COW 574		COW 575		COW 576	
		B‡	R§	B‡	R§	B‡	R§	B‡	R§
1*	Herd	3.7	3.69	3.6	3.58	3.1	3.09	3.0	2.98
2*	Herd	3.5	3.32	3.4	3.29	3.25	3.11	3.0	2.96
3*	Herd	3.5	3.38	3.6	3.45	3.2	3.06	3.2	3.03
4†	Herd	3.8	3.67	3.15	3.00	3.4	3.27	3.1	2.99
5†	Herd	3.6	3.47	3.45	3.31	3.5	3.34	2.9	2.89
6†	High oil	3.95	3.85	4.05	4.02	3.95	3.84	3.6	3.52
7†	High oil	3.4	3.19	3.9	3.84	3.2	2.98	3.0	2.84
8†	High oil	3.7	3.56	3.9	3.74	3.9	3.77	2.95	3.09
9†	High oil	3.9	3.66	3.5	3.24	3.35	3.16	3.2	3.15
10†	High oil	4.1	4.11	3.7	3.60	3.8	3.62	3.6	3.52
11†	High oil	3.05	2.79	3.7	3.54	3.5	3.28	3.0	3.85
12†	Herd	4.1	4.10	2.9	2.82	2.8	2.75	2.6	2.56
13†	Herd	3.1	3.01	3.2	3.10	3.1	3.01	2.8	2.55
14†	Herd	3.3	3.58	2.9	2.88	3.0	3.08	3.3	3.34
15†	Herd	3.2	3.17	3.3	3.30	3.05	3.08	2.5	2.39
16*	Herd	3.4	3.19	3.5	3.30	3.3	3.06	3.3	3.13
17*	Herd	3.3	3.19	3.5	3.28	3.4	3.21	3.1	3.03
18*	Herd	3.4	3.18	3.6	3.50	3.1	3.04	3.2	3.21
19†	Herd	3.6	3.58	3.5	3.46	3.2	3.09	3.0	2.98
20†	Herd	3.2	3.08	4.4	4.38	3.35	3.34	2.9	2.78
21†	High oil	3.1	3.09	4.3	4.25	3.4	3.22	3.8	3.76
22†	High oil	3.7	3.64	5.1	5.15	3.6	3.50	3.6	3.53
23†	High oil	4.2	4.11	5.0	4.92	3.6	3.28	3.1	3.07
24†	High oil	3.5	3.39	5.7	5.76	3.7	3.65	3.2	3.13
25*	High oil	3.85	3.68	3.6	3.50	3.5	3.35	3.1	2.94
26*	High oil	3.5	3.37	3.2	3.09	3.5	3.30	3.1	2.95
27*	High oil	3.5	3.47	3.2	3.13	3.25	3.22	3.05	2.99
28*	High oil	3.5	3.44	3.3	3.40	3.3	3.12	2.9	2.90
Average.........		3.55	3.46	3.72	3.61	3.37	3.24	3.11	3.07

* Composite sample preserved by formalin.

† Sample of single milking—no preservative.

‡ Babcock method, average of 2 determinations.

§ Roese-Gottlieb method, average of 2 determinations.

however, for cows 270 and 316, which had been in milk for a longer time, showed increases in test only slightly greater than cows 300 and 303.

It is believed that the failure of the soybean ration to cause a

TABLE 5

Summary of results

PERIOD	MILK, 7 DAYS	FAT, 7 DAYS	AVERAGE TEST (PER CENT FAT)	INCREASE IN TEST	INCREASE IN FAT YIELD

Series III, experiment A. Comparison of effect of soybean oil meal and soybeans on the percentage of fat in milk

Cow 262 (Guernsey)

	pounds	pounds		per cent	per cent
Soybean oil meal*.............	257.0	12.950	5.18		
Soybeans.....................	288.5	14.774	5.12	−2.1	−1.7
Soybean oil meal............	324.0	17.111	5.28		

Cow 270 (Guernsey)

Soybean oil meal*.............	167.2	9.671	5.87		
Soybeans.....................	165.4	10.155	6.14	6.1	5.2
Soybean oil meal............	168.8	9.637	5.70		

Cow 316 (Holstein)

Soybean oil meal*.............	276.5	9.880	3.57		
Soybeans.....................	266.2	9.643	3.64	4.6	−1.5
Soybean oil meal.............	286.1	9.703	3.39		

Cow 300 (Guernsey)

Soybean oil meal*.............	250.0	11.416	4.57		
Soybeans.....................	240.8	11.657	4.80	7.3	5.2
Soybean oil meal............	245.6	10.752	4.37		

Cow 303 (Guernsey)

Soybean oil meal*.............	122.7	6.579	5.36		
Soybeans.....................	135.0	7.099	5.26	3.2	2.3
Soybean oil meal............	150.2	7.299	4.83		

Cow 323 (Ayrshire)

Soybean oil meal*.............	173.1	8.844	5.11		
Soybeans.....................	176.3	8.699	4.93	3.0	9.3
Soybean oil meal.............	158.8	7.078	4.46		
Average periods 1 and 3:					
Soybean oil meal, 6 cows....	1,290.5	60.460	4.69		
Soybeans, 6 cows..........	1,271.2	62.027	4.88	4.1	2.6

* Period I was 13 days in length, but for comparative purposes has been calculated to a basis of 7 days.

TABLE 5—*Continued*

Series III, experiment B. Comparison of effect of linseed oil meal with flaxseed meal on the percentage of fat in milk

PERIOD	MILK, 7 DAYS	FAT, 7 DAYS	AVERAGE TEST (PER CENT FAT)	INCREASE IN TEST	INCREASE IN FAT YIELD

Series III, experiment B.   Comparison of effect of linseed oil meal with flaxseed meal on the percentage of fat in milk

Cow 262 (Guernsey)

	pounds	pounds		per cent	per cent
Linseed oil meal..............	331.9	15.782	4.76		
Flaxseed meal................	333.9	16.146	4.84	1.2	1.0
Linseed oil meal.............	336.8	16.193	4.81		

Cow 270 (Guernsey)

Linseed oil meal..............	163.5	9.281	5.68		
Flaxseed meal................	172.2	10.924	6.34	12.0	15.4
Linseed oil meal..............	171.0	9.650	5.64		

Cow 270 (Guernsey)

Linseed oil meal.............	141.3	8.126	5.75		
Flaxseed meal................	151.3	9.283	6.13	11.4	10.9
Linseed oil meal..............	163.6	8.614	5.26		

Cow 316 (Holstein)

Linseed oil meal..............	297.2	10.358	3.49		
Flaxseed meal................	293.8	11.142	3.80	7.6	11.2
Linseed oil meal.............	270.4	9.659	3.57		

Cow 300 (Guernsey)

Linseed oil meal..............	232.7	10.159	4.37		
Flaxseed meal................	227.5	11.343	4.98	11.7	13.2
Linseed oil meal..............	217.4	9.883	4.55		

Cow 303 (Guernsey)

Linseed oil meal..............	145.0	6.778	4.67		
Flaxseed meal................	146.6	7.341	5.01	9.2	6.4
Linseed oil meal..............	155.7	7.018	4.51		

Cow 323 (Ayrshire)

Linseed oil meal..............	153.8	6.834	4.44		
Flaxseed meal................	137.0	7.319	5.34	21.9	14.1
Linseed oil meal..............	138.6	5.991	4.32		

TABLE 5—*Concluded*

PERIOD	MILK, 7 DAYS	FAT, 7 DAYS	AVERAGE TEST (PER CENT FAT)	INCREASE IN TEST	INCREASE IN FAT YIELD

Series III experiment B. Comparison of effect of linseed oil meal with flaxseed meal on the percentage of fat in milk—Continued

Cow 6 (Grade Holstein)

	pounds	*pounds*		*per cent*	*per cent*
Flaxseed meal†	219.9	8.115	3.69		
Linseed oil meal	217.7	7.031	3.23		
Flaxseed meal	192.0	7.332	3.82	14.2	0.7
Linseed oil meal	217.4	7.529	3.46		

Cow 25 (Grade Holstein)

Flaxseed meal†	263.5	8.198	3.11		
Linseed oil meal	252.4	7.030	2.78		
Flaxseed meal	236.6	7.648	3.23	11.0	5.0
Linseed oil meal	247.5	7.544	3.04		
Average periods 1 and 3:					
Linseed oil meal, 9 cows	1,927.0	81.730	4.24		
Flaxseed meal, 9 cows	1,890.9	88.478	4.68	10.4	8.3

Series III, experiment C. Comparison of effect of peanut oil meal and peanuts on the percentage of fat in milk

Cow 316 (Holstein)

Peanut oil meal	262.1	9.573	3.65		
Peanuts	276.9	9.777	3.53	3.1	1.4
Peanut oil meal	297.3	9.719	3.20		

Cow 300 (Guernsey)

Peanut oil meal	195.9	8.596	4.39		
Peanuts	194.0	9.456	4.87	5.6	12.4
Peanut oil meal	170.2	8.224	4.83		

Cow 303 (Guernsey)

Peanut oil meal	155.9	6.891	4.42		
Peanuts	148.6	7.233	4.87	14.1	6.1
Peanut oil meal	163.9	6.745	4.12		

Cow 323 (Ayrshire)

Peanut oil meal	139.1	6.106	4.39		
Peanuts	135.3	6.888	5.09	18.6	15.7
Peanut oil meal	138.3	5.802	4.19		
Average periods 1 and 3:					
Peanut oil meal, 4 cows	761.4	30.828	4.05		
Peanuts, 4 cows	754.8	33.354	4.42	9.1	8.2

† Preliminary period.

greater effect upon the test was due to the comparatively small difference in the oil content of the soybean oil meal mixture and that containing the ground soybeans.

When flaxseed meal was substituted for linseed oil meal in amounts such that the concentrate mixture was as closely balanced to meet production requirements as before, greater effects upon the test were secured than with any other like substitution. The increases were as great as 21.9 per cent with an average for the nine records of eight cows of 11 per cent (table 5, experiment B). If cow 262 be omitted, the average would be about 12 per cent. The increase in the test was responsible for an increased fat yield for the week about 8 per cent greater than the average for the preceding and following weeks, although some of the increased fat yields were as much as 14 per cent. The milk yield was somewhat lower during the flaxseed meal period, the average for the group being 4.4 pounds per cow for the week, or about 0.6 pound per day.

The peanut oil meal and peanut mixtures were not consumed readily by some cows, and were refused entirely by others. Cow 270 was, therefore, again fed the rations used in experiment B, and her records included under that experiment. The substitution of peanuts for peanut oil meal gave increases in test of as much as 18 per cent, with an average of 9 per cent for the four cows (table 5, experiment C). Fat yields were also increased about 8 per cent.

Neither the peanut oil meal nor peanut mixtures were readily consumed, which is believed to be responsible for decreased milk production in several cases and failure to secure a greater effect upon the test.

The amounts of digestible nutrients consumed compared with the estimated requirements are shown in figures 5 and 10.

One of the interesting observations made during this series was that the test is not affected at once when the ration is changed from low oil to high oil, but that there is a latent period which seems to vary in length from 12 to 36 hours. Part of the records secured in Experiment B have been calculated in different ways in order to illustrate this point (table 6). It was

TABLE 6

Effect on production and test of changing from linseed oil meal ration to flaxseed ration

Records calculated in different ways*

COW NUMBER	CHECK PERIOD (4 DAYS PRECEDING FEED CHANGE)			RECORDS FOR ONE DAY BEGINNING WITH FEED CHANGE			RECORDS BEGINNING ONE DAY MILKING AFTER FEED CHANGE						RECORDS FOR 2 DAYS BEGINNING 2 MILKINGS AFTER FEED CHANGE				
							First day			First and second days							
	Average daily milk	Average daily fat	Fat	Milk	Fat	Fat	Milk	Fat	Fat	Average daily milk	Average daily fat	Fat	Average daily milk	Average daily fat	Fat	Increase in per cent fat	Increase in test per cent
	pounds	pounds	per cent	pounds	pounds	per cent	pounds	pounds	per cent	pounds	pounds	per cent	pounds	pounds	per cent		per cent
300	33.3	1.468	4.41	31.9	1.356	4.25	33.3	1.419	4.26	33.1	1.522	4.57	31.8	1.546	4.86	+0.45	10.2
262	47.2	2.145	4.55	44.7	2.034	4.55	48.3	2.144	4.44	47.3	2.275	4.81	47.5	2.294	4.84	+0.29	6.4
270	23.5	1.395	5.94	22.9	1.365	5.95	24.5	1.541	6.29	24.4	1.609	6.50	24.5	1.643	6.70	+0.76	12.8
270	20.6	1.176	5.71	20.6	1.175	5.70	21.0	1.333	6.35	21.5	1.395	6.50	21.8	1.462	6.70	+0.99	17.3
303	19.7	0.938	4.75	21.1	0.982	4.65	20.1	0.993	4.94	19.4	0.978	5.04	19.9	0.996	5.00	+0.25	5.3
316	42.2	1.465	3.47	43.4	1.504	3.46	42.5	1.528	3.59	42.5	1.638	3.86	42.7	1.685	3.95	+0.48	13.8
323	21.6	0.954	4.42	22.4	0.969	4.33	22.6	0.968	4.28	22.5	1.044	4.64	22.9	1.123	4.89	+0.47	10.7
Average...	29.7	1.363	4.59	29.6	1.341	4.53	30.3	1.418	4.68	30.1	1.494	4.98	30.2	1.536	5.09	+0.50	10.9
Average gain or loss......				−0.1	−0.022		+0.6	+0.055		+0.4	+0.131	+0.39	+0.5	+0.173	+0.50	+0.50	
Increase in test, per cent...						−1.3			2.0			8.5					10.9

* Cows milked twice daily, except cow 316. Records for cow 316 calculated for same periods as other cows.

found that when the milk yields and fat yields were computed for one day beginning with the milking coinciding with the change in feed, that the test was no higher than in the four day check period preceding the change. Records for one day and for two days beginning one milking after the feed change were tabulated. The first days' test showed an increase of 2 per cent above that of the check period, while the increase in test for the two days was 8.5 per cent, thus indicating that the change during the second day was greater than during the first. When two milkings after the feed change were omitted, the records for the two following days showed an increase in test of nearly 11 per cent.

Another point brought out in these studies was that the high oil ration has a residual effect lasting from 12 to 24 hours or more (table 7). In the latter table, the records are given by periods beginning one day after the changes in rations were made. Further studies of the latent effects of feed are in progress.

In view of the increases in test obtained in these experiments in which concentrate mixtures containing about 11.5 per cent digestible protein were fed, the question was raised as to whether similar increases could be obtained with rations higher in protein. Accordingly, cow 270 was fed for three additional periods of seven days each. During the first and third periods, a "test cow mixture," consisting of corn, oats, wheat bran, linseed oil meal, cottonseed meal, soybean oil meal and gluten meal, and containing about 17 per cent digestible protein, was fed. During the second period, flaxseed meal was substituted for a part of the test mixture. The detailed production records are shown in table 7.

The data show that the daily yield of fat and the test were approximately 16 per cent greater during the flaxseed period than during the test ration periods.

Series IV

The procedure during Series IV was similar to that of the preceding series, except that each experiment was extended to include two more weeks, during one of which oil plus oil meal

TABLE 7

The effect of feeding flaxseed when the test ration is used as the basic ration. Flaxseed substituted for part of test ration during flaxseed period

Guernsey cow no. 270

	MILK YIELD		FAT	FAT YIELD	
	Per milking	Daily		Per milking	Daily
Test ration					
	pounds	*pounds*	*per cent*	*pounds*	*pounds*
May 10—A	11.0		5.3	0.583	
P	10.5	21.5	5.4	0.567	1.150
May 11—A	11.3		5.6	0.633	
P	10.2	21.5	5.4	0.551	1.184
May 12—A	11.6		5.3	0.615	
P	10.2	21.8	5.6	0.571	1.186
May 13—A	12.5		5.4	0.675	
P	12.7	25.2	5.3	0.673	1.348
May 14—A	13.4		5.6	0.750	
P	9.6	23.0	5.8	0.557	1.307
May 15—A	11.9		5.8	0.690	
P	10.0	21.9	6.0	0.600	1.290
May 16—A	11.0		4.7	0.517	
P	10.0	21.0	6.5	0.650	1.167
Total		155.9			8.632
Average		22.3	5.56		1.277
Flaxseed ration					
	pounds	*pounds*	*per cent*	*pounds*	*pounds*
May 17—A	12.1		5.7	0.690	
P	9.9	22.0	6.2	0.614	1.304
May 18—A	11.2		6.0	0.672	
P	9.8	21.0	6.3	0.617	1.289
May 19—A	11.5		6.3	0.725	
P	9.6	21.1	6.8	0.653	1.378
May 20—A	12.6		6.4	0.806	
P	10.3	22.9	7.4	0.762	1.568
May 21—A	11.8		6.9	0.814	
P	10.5	22.3	7.4	0.777	1.591
May 22—A	11.6		5.9	0.684	
P	9.9	21.5	7.2	0.713	1.397
May 23—A	12.2		6.7	0.817	
P	10.0	22.2	7.3	0.730	1.547
Total		153.0			10.074
Average		21.9	6.58		1.439

TABLE 7—*Continued*

	MILK YIELD		FAT	FAT YIELD	
	Per milking	Daily		Per milking	Daily
Test ration					
May 24—A	10.4		6.5	0.676	
P	9.6	20.0	6.9	0.661	1.337
May 25—A	12.5		6.5	0.813	
P	10.7	23.2	6.0	0.642	1.455
May 26—A	10.8		5.6	0.605	
P	9.8	20.6	5.3	0.519	1.124
May 27—A	12.5		5.4	0.675	
P	9.9	22.4	5.4	0.535	1.210
May 28—A	11.8		5.2	0.614	
P	10.1	21.9	5.8	0.586	1.200
May 29—A	12.0		5.6	0.672	
P	9.2	21.2	5.5	0.506	1.178
May 30—A	12.3		5.7	0.701	
P	9.9	22.2	5.6*	0.554	1.256
Total		151.5			8.760
Average		21.6	5.78		1.251

	MILK	FAT	
	pounds	*pounds*	*per cent*
Average daily production on test ration	22.0	1.242	5.66
Average daily production on flaxseed ration	21.9	1.439	6.58

Increase in yield of fat, per cent................................... 15.9
Increase in test, per cent... 16.3

* Sample lost. Average test of three previous p.m. milkings used.

characterized the ration. The rations differed from those of Series III in being less bulky and thus more readily consumed. Corn, oats and corn starch were used instead of the large quantities of beet pulp, these feeds being used to provide energy and to keep at a low level the protein and oil content of that part of the concentrate mixture other than the feeds being compared.

On account of the differences in the requirements for the production of milk containing different percentages of fat, two separate concentrate mixtures were formulated for each period, one being calculated for Holsteins and Ayrshires, and the other

TABLE 8

Summary of results

PERIOD	MILK, 7 DAYS	FAT, 7 DAYS	AVERAGE TEST (PER CENT FAT)	INCREASE IN TEST	INCREASE IN FAT

Series IV, experiment A. Comparison of effect of linseed oil meal, flaxseed meal and linseed oil meal plus linseed oil on the percentage of fat in milk

Cow 299 (Jersey)

	pounds	pounds		per cent	per cent
Linseed oil meal...............	200.7	9.988	4.98		
Flaxseed meal................	204.0	10.475	5.13	8.7	6.9
Linseed oil meal..............	215.9	9.606	4.45		
Linseed oil meal + linseed oil.	215.0	10.185	4.74	9.5	10.9
Linseed oil meal..............	208.3	8.768	4.21		

Cow 340 (Holstein)

Linseed oil meal..............	245.7	7.485	3.05		
Flaxseed meal................	233.5	6.988	2.99	0.7	−0.01
Linseed oil meal..............	224.3	6.493	2.89		
Linseed oil meal + linseed oil.	221.1	7.022	3.18	15.6	13.3
Linseed oil meal..............	226.4	5.905	2.61		

Cow 288 (Holstein)

Linseed oil meal..............	352.0	11.415	3.24		
Flaxseed meal................	341.7	10.367	3.03	1.2	−0.5
Linseed oil meal..............	342.0	9.412	2.75		
Linseed oil meal + linseed oil.	342.3	10.527	3.08	13.0	15.5
Linseed oil meal..............	327.0	8.819	2.70		

Cow 288

Linseed oil meal..............	314.9	10.043	3.189		
Linseed oil meal..............	312.3	9.619	3.080		
Flaxseed meal................	306.5	10.400	3.39	10.1	9.7
Linseed oil meal..............	300.4	9.077	3.02		
Linseed oil meal..............	303.8	9.181	3.02		

Average periods 1 and 3:....					
Linseed oil meal, 3 cows...	790.3	27.200	3.44		
Flaxseed meal, 3 cows.....	779.2	27.830	3.57	3.7	2.3
Average periods 3 and 5:					
Linseed oil meal, 3 cows...	772.0	24.502	3.17		
Linseed oil meal + linseed					
oil, 3 cows..............	778.4	27.734	3.56	12.3	13.2

TABLE 8—*Continued*

PERIOD	MILK, 7 DAYS	FAT, 7 DAYS	AVERAGE TEST (PER CENT FAT)	INCREASE IN TEST	INCREASE IN FAT

Series IV, experiment B. Comparison of effect of peanut oil meal, peanuts, and peanut oil meal plus peanut oil on the percentage of fat in milk

Cow 270 (Guernsey)

	pounds	pounds		per cent	per cent
Peanut oil meal...............	204.9	10.424	5.087		
Peanuts......................	201.4	11.415	5.668	13.6	8.6
Peanut oil meal...............	216.4	10.590	4.89		
Peanut oil meal + peanut oil..	193.0	10.978	5.69	16.0	7.8
Peanut oil meal...............	198.5	9.773	4.92		

Cow 340 (Holstein)

Peanut oil meal...............	233.5	6.551	2.805		
Peanuts......................	214.8	7.067	3.290	14.9	8.1
Peanut oil meal...............	223.2	6.518	2.92		
Peanut oil meal + peanut oil..	214.7	6.772	3.15	12.3	6.4
Peanut oil meal...............	232.4	6.266	2.69		

Cow 299 (Jersey)

Peanut oil meal...............	221.6	10.167	4.588		
Peanuts......................	206.8	10.051	4.860	9.7	3.0
Peanut oil meal...............	219.2	9.357	4.27		
Peanut oil meal + peanut oil..	213.5	10.408	4.87	16.7	14.5
Peanut oil meal...............	216.4	8.819	4.07		

Cow 334 (Jersey)

Peanut oil meal..............	232.0	11.377	4.904		
Peanuts......................	234.9	11.481	4.888	7.0	1.2
Peanut oil meal...............	264.7	11.312	4.27		
Peanut oil meal + peanut oil..	238.2	11.283	4.73	5.3	−1.8
Peanut oil meal...............	246.3	11.657	4.73		

Cow 323 (Ayrshire)

Peanut oil meal...............	226.6	10.532	4.648		
Peanuts......................	224.7	9.690	4.312	−0.9	−0.4
Peanut oil meal...............	222.5	9.003	4.07		
Peanut oil meal + peanut oil..	219.3	9.248	4.26	14.8	7.2
Peanut oil meal...............	224.8	8.244	3.66		
Average periods 1 and 3:					
Peanut oil meal, 5 cows...	1,132.3	47.916	4.23		
Peanuts, 5 cows..........	1,082.6	49.704	4.59	8.5	3.7
Average periods 3 and 5:					
Peanut oil meal, 5 cows...	1,132.2	45.770	4.04		
Peanut oil meal + peanut					
oil.....................	1,078.7	48.689	4.51	11.64	6.4

TABLE 8—*Continued*

PERIOD	MILK, 7 DAYS	FAT, 7 DAYS	AVERAGE TEST (PER CENT FAT)	INCREASE IN TEST	INCREASE IN FAT

Series IV, experiment C.   Comparison of effect of soybean oil meal, soybeans, and soybean oil meal plus soybean oil on the percentage of fat in milk

Cow 270 (Guernsey)

	pounds	pounds		per cent	per cent
Soybean oil meal..............	193.5	9.637	4.980		
Soybeans.....................	178.7	9.652	5.401	4.4	0.1
Soybean oil meal..............	179.6	9.656	5.376		
Soybean oil meal + soybean oil.........................	167.0	9.175	5.494	1.5	−5.2
Soybean oil meal	167.3	9.699	5.797		

Cow 340 (Holstein)

Soybean oil meal..............	213.8	6.123	2.864		
Soybeans.....................	209.9	6.249	2.977	1.1	−5.1
Soybean oil meal..............	233.7	7.055	3.019		
Soybean oil meal + soybean oil.........................	233.1	6.649	2.852	−5.7	−4.7
Soybean oil meal	227.6	6.899	3.031		

Cow 288 (Holstein)

Soybean oil meal..............	292.4	8.994	3.076		
Soybeans.....................	295.9	9.541	3.224	−3.1	6.1
Soybean oil meal..............	283.3	8.995	3.175		
Soybean oil meal + soybean oil.........................	300.7	9.188	3.056	−3.0	1.1
Soybean oil meal..............	290.5	9.174	3.158		

Cow 299 (Jersey)

Soybean oil meal..............	212.5	9.519	4.480		
Soybeans.....................	195.4	8.740	4.473	−4.7	−7.7
Soybean oil meal.............	191.4	9.422	4.923		
Soybean oil meal + soybean oil.........................	197.9	9.485	4.793	−0.8	1.1
Soybean oil meal..............	196.8	9.341	4.746		

Cow 323 (Ayrshire)

	pounds	pounds		per cent	per cent
Soybean oil meal.............	203.3	7.722	3.798		
Soybeans.....................	195.7	8.012	4.094	5.7	4.3
Soybean oil meal..............	193.5	7.643	3.950		
Soybean oil meal + soybean oil	175.3	6.964	3.973	−8.0	−11.1
Soybean oil meal..............	173.6	8.021	4.620		

TABLE 8—*Concluded*

Series IV, experiment C. Comparison of effect of soybean oil meal, soybeans and soybean oil meal plus soybean oil on the percentage of fat in milk

Cow 323 (Ayrshire)—*Continued*

PERIOD	MILK, 7 DAYS	FAT, 7 DAYS	AVERAGE TEST (PER CENT FAT)	INCREASE IN TEST	INCREASE IN FAT
				per cent	per cent
Average periods 1 and 3:					
Soybean oil meal, 5 cows....	1,098.5	42.383	3.86		
Soybeans, 5 cows	1,075.6	42.194	3.92	1.6	−0.4
Average periods 3 and 5:					
Soybean oil meal, 5 cows....	1,068.7	42.953	4.02		
Soybean oil meal + soybean oil, 5 cows...............	1,074.0	41.461	3.86	−4.0	−3.5

for Jerseys and Guernseys. The experiments began shortly after the cows had calved, the stage of lactation being as follows: Cow 299, 44 days; cow 340, 34 days; cow 288, 37 days; cow 270, 37 days; cow 334, 10 days; cow 323, 21 days.

Since experiments of Series III had shown a latent period and a residual effect of the high oil feeding, the records of milk production during Series IV were calculated for periods beginning one day after the changes in the rations were made.

The comparison of the oil meal feeds with the kind of seeds from which the oil meals were derived gave results similar to those of Series III (table 8). Flaxseed meal, however, did not affect the test to so great an extent as before. During period IV of experiment A, linseed oil was administered to cows 299, 340 and 288, in amounts of 1.5, 1 and 1.7 pounds, respectively. The linseed oil apparently had greater effects upon the test than the feeding of an equal amount of oil in the flaxseed meal rations. The few records obtained, however, can not be taken as indicating the exact extent of the effects upon the test to be expected from changes of this sort in the rations.

The comparison of peanut oil meal and peanuts was more satisfactory than before because the feeds were more palatable. The peanut oil meal was quite readily consumed, but the peanuts, at first fed raw and later roasted, were not eaten so readily as the oil meal. The increases in test due to the peanut feeding (table

8, experiment B) were similar to those obtained in Series III, while the peanut oil gave slightly greater increases. The oil was fed in amounts ranging from 1.6 to 2.6 pounds per head daily.

The comparison of soybean oil meal with soybeans, and the addition of soybean oil to soybean oil meal concentrate mixtures, showed practically no effect of high oil rations upon the test of milk. It is believed that the primary reason for this as suggested above, was the small differences between the low oil and high oil rations. While some of the cows received as much as 2.5 to 3.0 pounds of digestible fat daily during the high oil periods in experiments A and B, the amounts were not over 1.4 pounds daily during the soybean comparisons. The amounts of soybean oil fed ranged from 0.3 to 0.56 pound daily.

A factor which seemed to influence feed consumption and milk yields during experiment C, although it may have had no influence upon the test, was the unusually hot weather for the season of the year (May 2 to June 6, 1925).

Increases in test like those obtained as a result of the flaxseed and peanut feeding would be of much significance if obtained during the taking of an "official test," because the amount of butterfat credited to a cow might be materially increased as a result of the higher fat percentages. Perhaps of even greater importance is the higher percentage of fat which might be obtained during a one-day or two-day "semi-official test," for the average percentage of fat obtained during the one-day or two-day period forms the basis for the calculation of the butterfat yields for an entire month. A cow might thus be credited with a yield of fat greatly in excess of her actual production.

Another point of interest in connection with the making of official tests, is the effect of linseed oil upon the test of the milk. Linseed oil is sometimes administered as a laxative or physic, and might be so used during an official test. It is shown in these experiments that the consumption of linseed oil (given by mouth) increased the test of the milk.

These series of experiments indicate that the composition of milk can be materially influenced by changes in the feed which

increase the amount of digestible fat or oil. The feeding of oils extracted from seeds of different plants had an effect upon the test of the milk similar to that produced by feeding the seeds. It is believed that possible stimulating effects due to changes in protein intake and to the introduction of feeds from different plant sources were absent from the experiments of Series III and IV.

Further, in Series IV, differences in the energy content of the rations, calculated in terms of digestible nutrients, were largely avoided, so that increases in the test of the milk could not be due to a stimulating effect caused by a large surplus of energy above the requirements. It is logical to conclude, therefore, that the factor causing changes in the percentage fat content of the milk was the differences in oil content of the rations compared.

PALATABILITY OF FEEDS

Several of the mixtures fed were not relished, making it necessary to weigh and account for the orts or to change the character of the rations. Production was not so liberal on some rations as it might have been, had the feed been consumed more freely.

Difficulty was encountered in Series III in securing complete consumption of the concentrate mixtures. This was partly due to great bulkiness caused by the large quantities of soaked beet pulp, although the lack of palatability of some of the feeds was also responsible.

The peanut oil meal used in Series III had a sharp, burned odor and taste and was refused by some cows, while in Series IV it was of much better quality. The peanuts fed were shelled No. 2 Virginia peanuts, and were fed both raw and roasted, ground and unground. Some cows refused to eat peanuts, while the ones which did eat them seemed to relish them roasted better than in the raw state. As many as 6.5 pounds of peanuts were consumed per cow daily.

Soybeans did not prove as palatable as the soybean oil meal. About 4 pounds of soybeans daily per cow was the maximum amount fed.

Flaxseed meal was eaten readily by all the cows, and as much

as 45 pounds weekly was fed without apparent disturbance of the body functions.

Peanut oil and soybean oil were consumed completely when mixed with the concentrates, but linseed oil was refused and was given as a drench.

SUMMARY AND CONCLUSIONS

Experiments were carried out to determine the effect of rations high in oil content upon the composition of milk, with particular reference to its percentage fat content (test).

The general plan of procedure followed was to feed rations low in oil content and high in oil content in alternate periods, and to compare the results obtained during a high oil period with the average of the results of the low oil periods immediately preceding and following it.

The feeding of mixtures of sunflower seed, soybean seed and high oil corn did not seem to stimulate milk production and effects upon the test, if any, were not evidenced in weekly composite samples of the milk.

In the second series of experiments, mixtures of sunflower seed, high oil corn and soybean seed caused increases in the test of the milk of 8 to 18 per cent as compared to the tests during periods when the usual herd ration was fed. Slight changes in the percentage of total solids followed in the same direction as the test.

In the third series, all concentrate mixtures contained the same percentage of digestible protein, and the mixtures were fed at a level intended to meet the estimated requirements closely. The mixtures fed during periods I and III of experiments A, B and C contained soybean oil meal, linseed oil meal and peanut oil meal, respectively, while during period II of the same experiments the corresponding oil meals were replaced by soybeans, flaxseed and peanuts. The increases in test secured as a result of the soybean feeding compared to the soybean oil meal periods were small or negative, while flaxseed gave increases of as much as 20 per cent, with an average of over 10 per cen- for nine records, as compared with linseed oil meal. The peat

nuts gave increases of as much as 18 per cent, with an average of 9 per cent for four cows, as compared with peanut oil meal. Fat yields were increased by both flaxseed and peanuts to the extent of about 8 per cent.

In Series IV, the rations were carefully balanced and fed to meet requirements closely. As in the preceding series, a com-
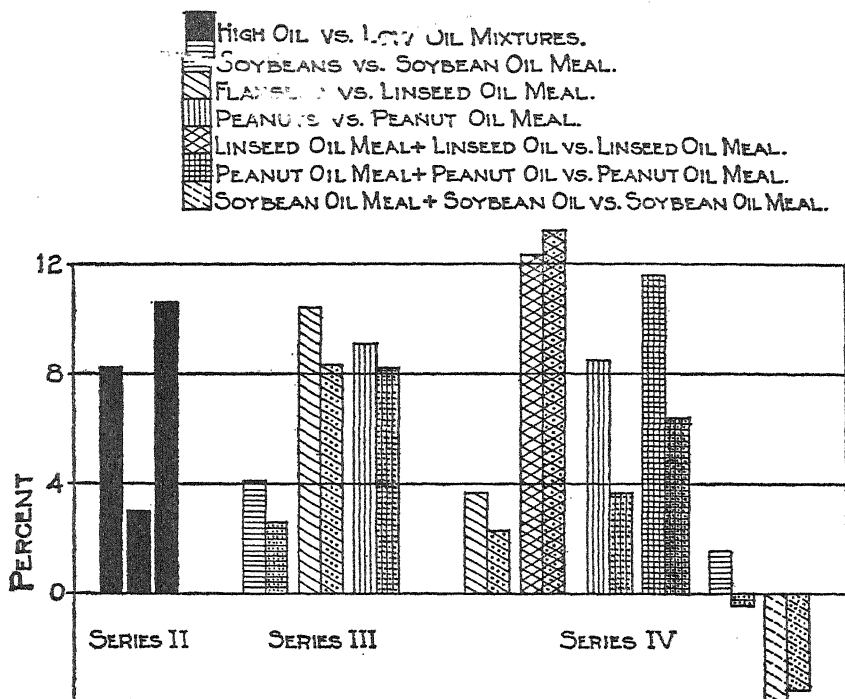
FIG. 1. GRAPHIC SUMMARY OF RESULTS OBTAINED IN THE COMPARISONS OF "HIGH OIL" WITH "LOW OIL" RATIONS

The heights of the columns represent the percentage increases obtained. The columns in Series II and the left sides of the double columns in Series III and IV, represent the percentage increases in test, whereas the dotted right sides of the double columns represent the percentage increases in yields of fat.

parison of soybeans with soybean oil meal showed little effect, but there were distinct increases in the test due to flaxseed feeding compared with linseed oil meal and to feeding peanuts in

contrast to peanut oil meal, although these were of less magnitude than before and in one or two cases were small or negative. The feeding of linseed oil and peanut oil as components of rations containing linseed oil meal and peanut oil meal, respectively, caused increases of about 12 per cent in the test of the milk as compared to iso-dynamic oil meal rations. Fat yields were in. ... about 13 per cent by the linseed oil feeding and about 6 per cent by the peanut oil feeding.

It is believed that these experiments establish that (a) the percentage fat composition of milk can be materially increased by the feeding of rations high in oil as compared with rations low in oil but furnishing an equal amount of total digestible nutrients; (b) the increase can be induced by the feeding of ground whole seeds such as peanuts and flaxseed, or by the oils extracted from these seeds; (c) the factor responsible for the increase in test is the oil itself and not a stimulation due to a large excess of energy in the rations, nor to a so-called specific effect of any of the feeds used other than that which may be closely associated with the oil.

It was noted that changing from a low oil to a high oil ration is not followed at once by an effect upon the test, but that there is a latent period of from 12 to 36 hours before the effect is produced. In changing from a high oil to a low oil ration there is a residual effect of the high oil feed lasting about the same length of time.

The length of time over which an increase in the test can be sustained by rations high in oil was not determined, but the data obtained indicate that as a rule, the greatest effect is obtained within the first two to three days and that the test then tends to decline.

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APPENDIX

Note: In the charts which follow, economy of space has been sought by superimposing one column upon another. It is assumed that the bases of all columns rest upon the horizontal line at the zero point of the scale. The amounts of digestible protein and total digestible nutrients may thus be read directly from the scale at the left. The amounts of digestible fat may likewise be read in the same manner, but in order to make these visible in the charts, the actual amounts consumed were multiplied by ten in figures 5 to 7, and by six in the remainder.
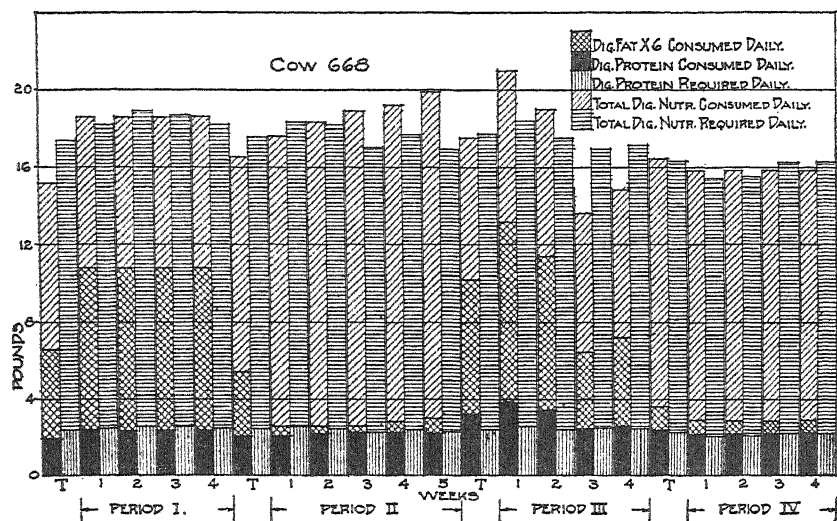
FIG. 2. COMPARISON OF DIGESTIBLE NUTRIENT CONSUMPTION AND ESTIMATED
REQUIREMENTS OF COW 66S DURING SERIES I
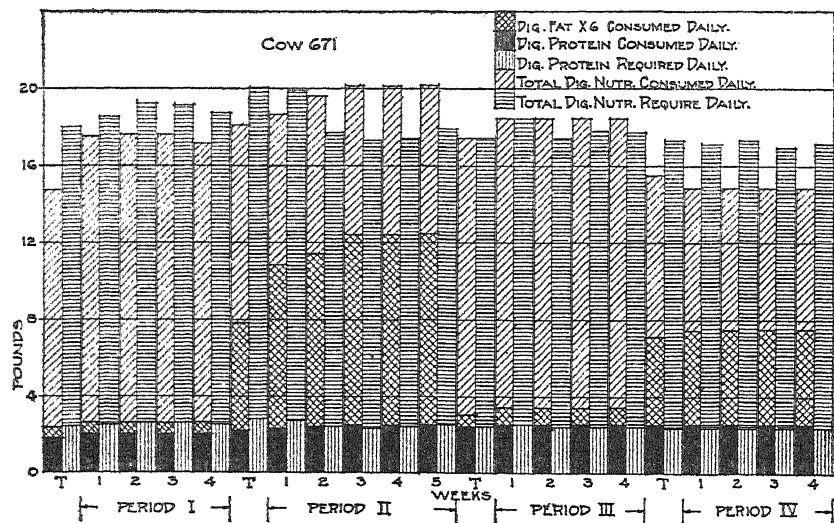
FIG. 3. COMPARISON OF DIGESTIBLE NUTRIENT CONSUMPTION AND ESTIMATED
REQUIREMENTS OF COW 671 DURING SERIES I
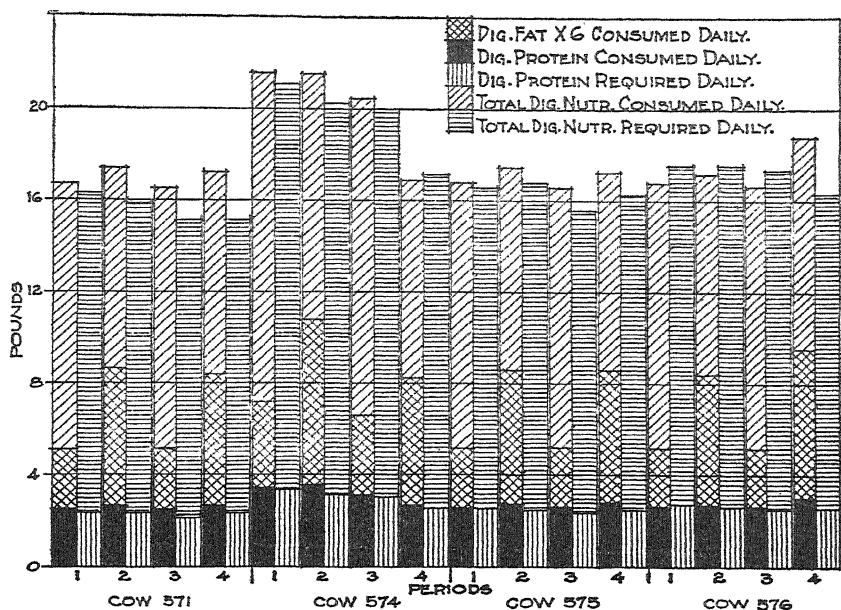
Fig. 4. Comparison of Digestible Nutrient Consumption and Estimated Requirements of Cows during Series II
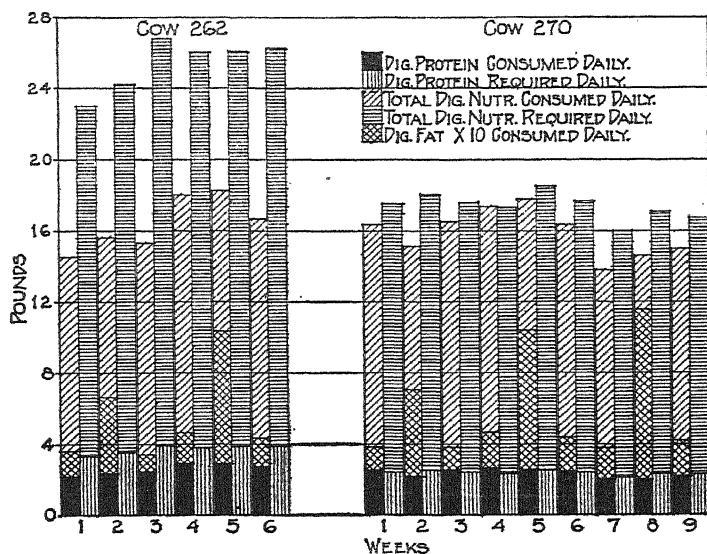
Fig. 5. Comparison of Digestible Nutrient Consumption and Estimated Requirements of Cows 262 and 270 during Series III

339
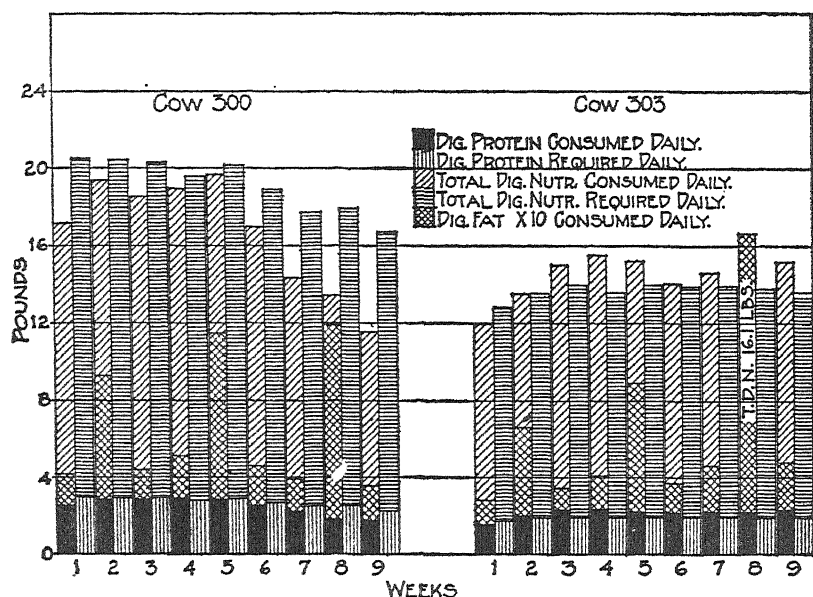
Fig. 6. Comparison of Digestible Nutrient Consumpt. ν and Estimated Requirements of Cows 300 and 303 during Series III
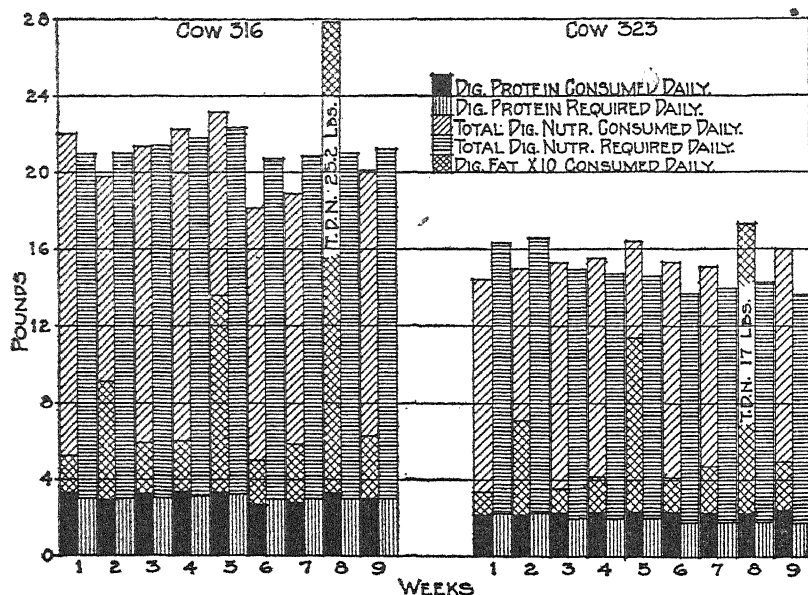
Fig. 7. Comparison of Digestible Nutrient Consumption and Estimated Requirements of Cows 316 and 323 during Series III
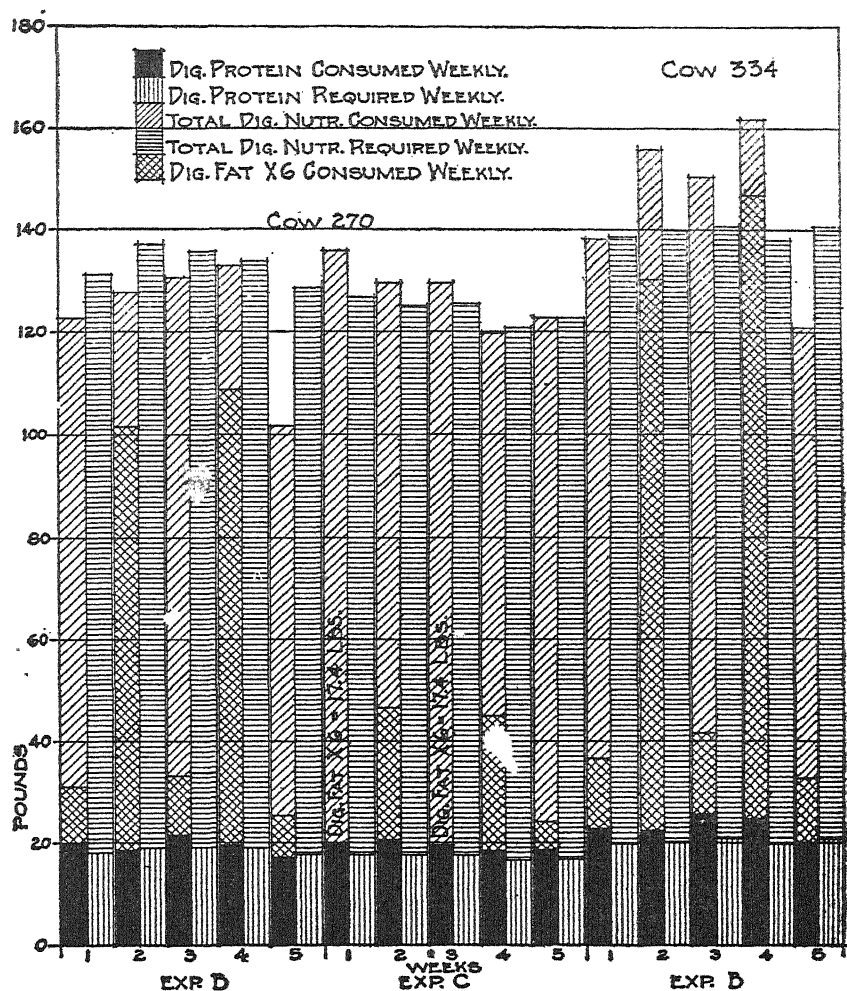
FIG. 8. COMPARISON OF DIGESTIBLE NUTRIENT CONSUMPTION AND ESTIMATED REQUIREMENTS OF COWS 270 AND 334 DURING SERIES IV
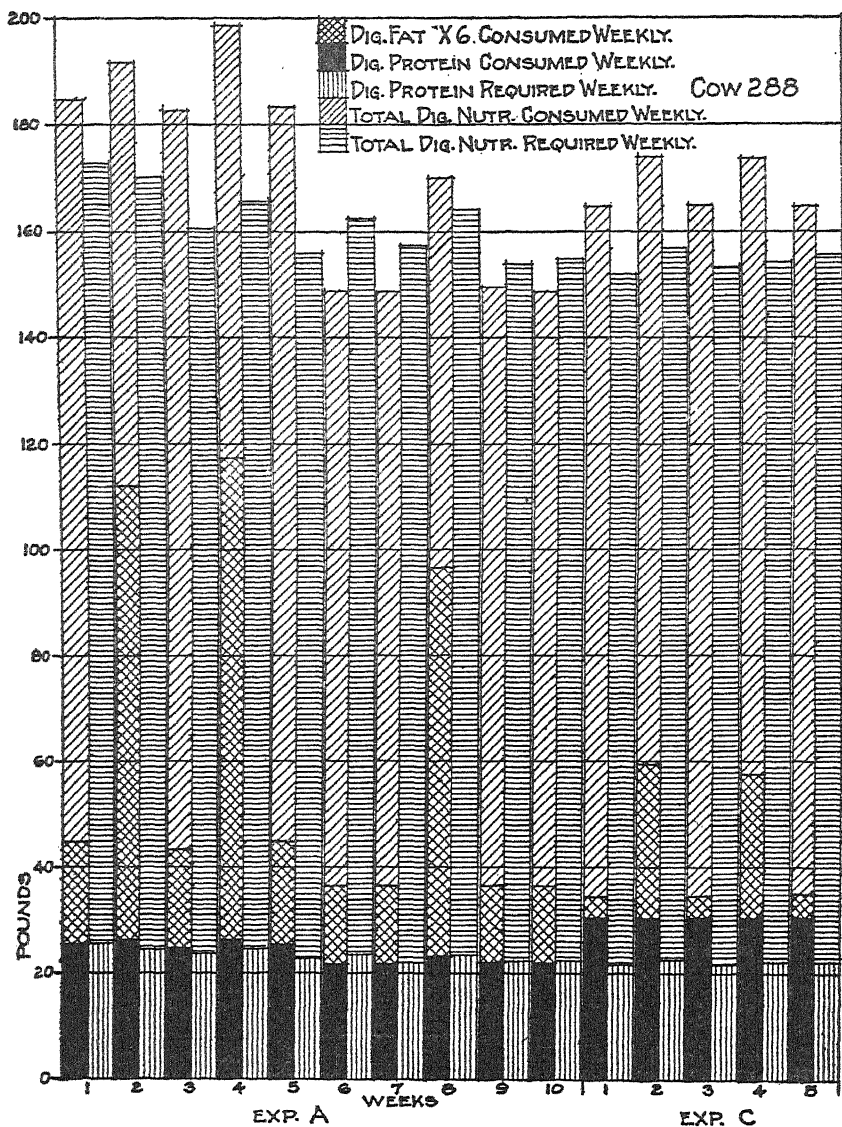
FIG. 9. COMPARISON OF DIGESTIBLE NUTRIENT CONSUMPTION AND ESTIMATED REQUIREMENTS OF COW 288 DURING SERIES IV
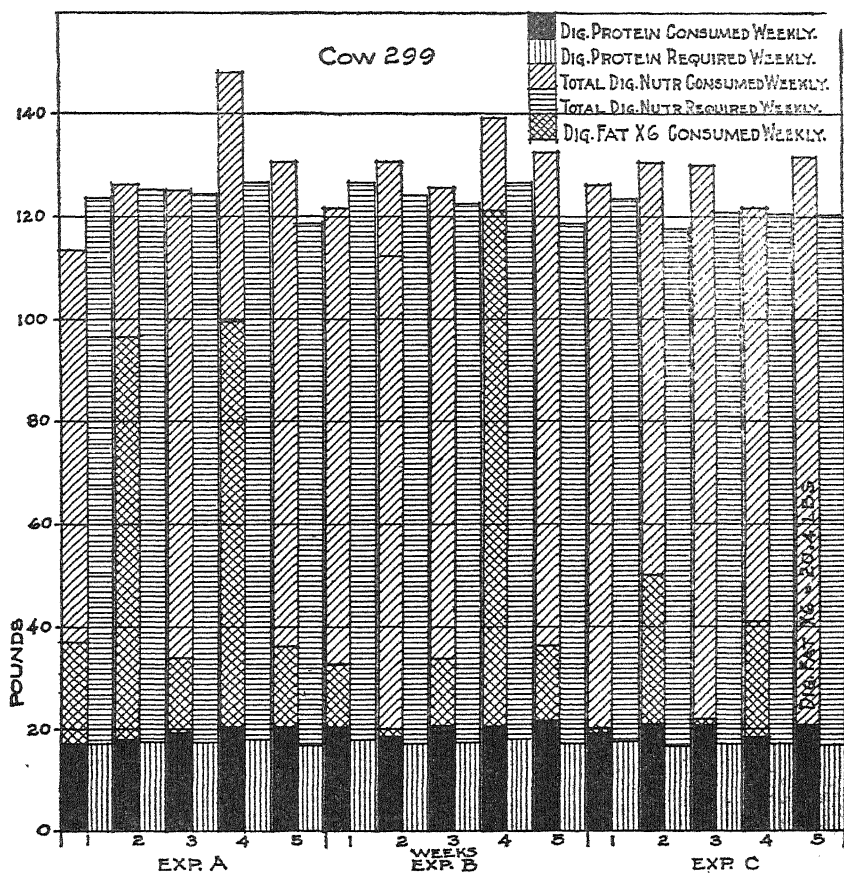
FIG. 10. COMPARISON OF DIGESTIBLE NUTRIENT CONSUMPTION AND ESTIMATED REQUIREMENTS OF COW 299 DURING SERIES IV
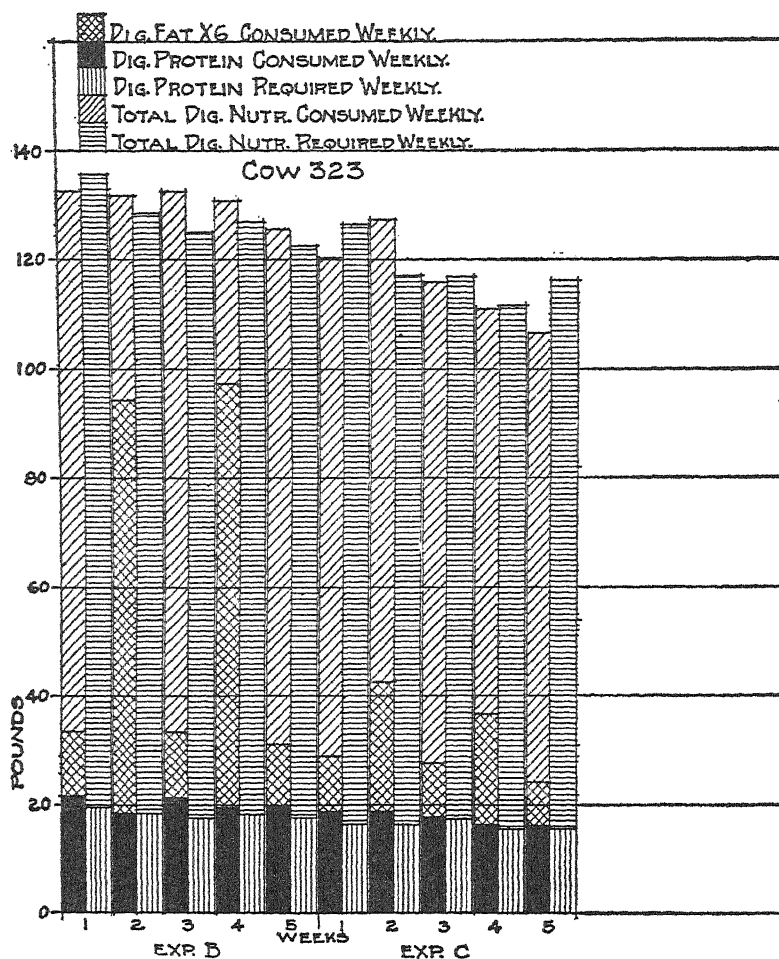
FIG. 11. COMPARISON OF DIGESTIBLE NUTRIENT CONSUMPTION AND ESTIMATED REQUIREMENTS OF COW 323 DURING SERIES IV
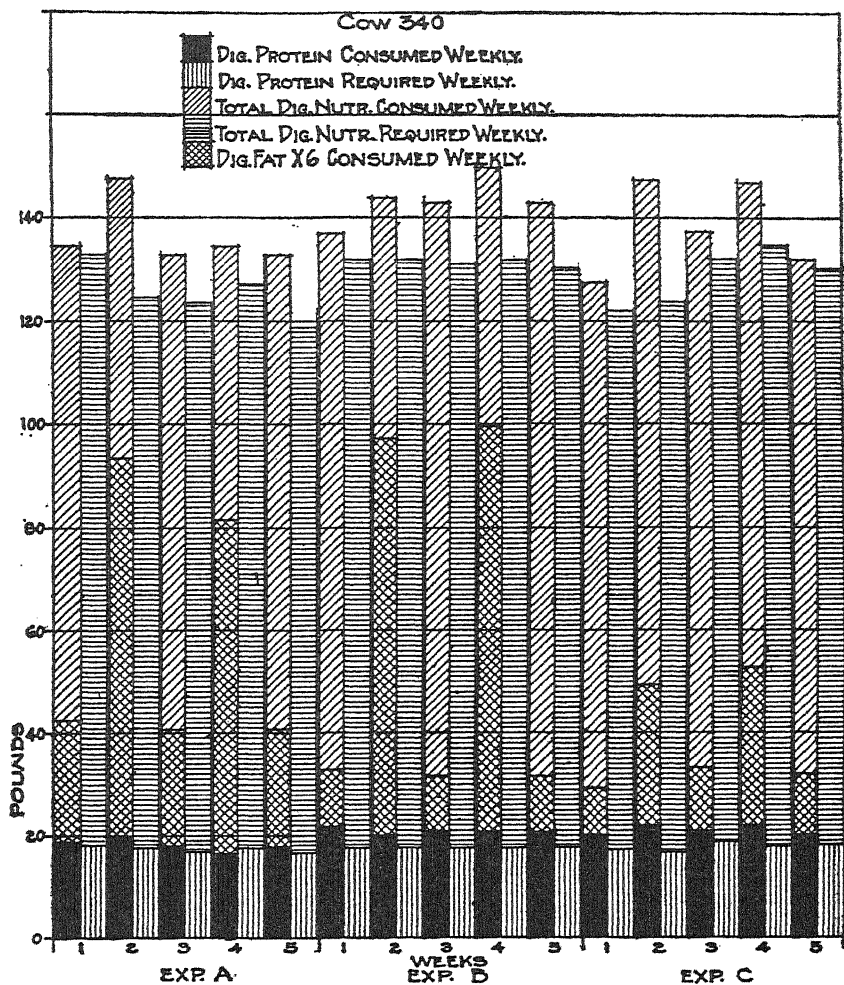
FIG. 12. COMPARISON OF DIGESTIBLE NUTRIENT CONSUMPTION AND ESTIMATED REQUIREMENTS OF COW 340 DURING SERIES IV

SHRINKAGE OF PRINT BUTTER*

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One of the most annoying considerations in handling butter is shrinkage, which makes it difficult to maintain legal weights. This is particularly true when it is held in cold storage for several months and when there has been sufficient time for evaporation of moisture. Shrinkage of butter is largely loss of water, and the decrease in moisture content is due mostly to evaporation.

This phenomenon is accelerated when the moisture is not thoroughly mixed with the fat. It is decreased when these two components of butter are so thoroughly worked together that the moisture droplets are small and thoroughly encased in fat. It is, therefore, apparent that the evaporation of moisture from butter, which may result in a shrinkage in weight below the legal limit, can be one of the butter dealer's most perplexing problems. The amount of working that butter receives probably has more to do with this loss of moisture than has any other factor.

In 1921 the author made a study of the effect of different types of tin-foil wrappers for one-pound butter prints on the keeping quality and the shrinkage of butter. It was easy at that time to obtain additional data on the loss in weight by evaporation when the butter was worked only sufficiently to prevent mottles and on additional working to better incorporate the moisture.

The butter was the regular make of sweet-cream goods in the Cornell University creamery. The Victor churn with one set of rolls was used. At the usual time of 50 revolutions of the churn in the working process, all of the butter was removed from the churn with the exception of one 90-pound Friday boxful. This butter was worked 18 additional revolutions. Seventy-five prints of the butter worked 50 revolutions and seventy-six prints of the butter worked 68 revolutions were put in the experiment.

The prints were weighed on a Torsion butter scale which is graduated to read in thirty-seconds of an ounce. It was weighed on October 27, November 19, November 26, December 10, December 24, January 7, January 20, February 11, March 2,

TABLE 1

Effect of working on shrinkage of butter

SAMPLE	OCTOBER 28 TO NOVEMBER 5	NOVEMBER 12	NOVEMBER 19	NOVEMBER 26	DECEMBER 10	DECEMBER 24	JANUARY 7	JANUARY 20	FEBRUARY 11	MARCH 2	APRIL 10	NUMBER OF PRINTS	TOTAL LOSS*	AVERAGE LOSS* PER PRINT
Division 1. Worked 50 revolutions of Victor churn														
A	−23*	+1	−3	−5	0	−4	−8	−5	−2	0	−27	12	76	6.33
B	−23	−8	−3	0	−8	−8	−9	−4	−10	0	−8	13	81	6.23
C	−12	−1	−23	−2	−4	−5	−18	+2	−2	−21	−1	13	87	6.69
D	−13	−6	−2	−8	−4	−9	−15	−3	−8	0	−9	13	77	5.92
E	−8	−12	+3	−20	−8	−6	−16	0	−10	0	−20	12	97	8.08
F	−14	+37	−13	−7	−16	−1	−14	−2	−19	−4	−19	12	72	6.00
Total...	−93	+11	−41	−42	−40	−33	−80	−12	−51	−25	−84	75	490	6.54
Division 2. Worked 68 revolutions of Victor churn														
A′	−7	−8	+19	−16	−6	−5	−8	−7	+1	−13	−7	13	57	4.38
B′	+2	+8	−5	−17	0	−9	+5	−10	−19	−5	−3	13	53	4.08
C′	−5	+3	−2	−3	−2	−18	+10	+11	−11	−1	−16	12	34	2.83
D′	−12	−14	+6	+2	−1	−2	−3	−21	−8	−2	−3	13	58	4.46
E′	+6	−11	−10	−24	+1	−9	−9	−4	+29	−21	−14	13	66	5.07
F′	−3	−1	+1	−16	−8	+2	−12	−6	−2	−15	+2	12	58	4.83
Total...	−19	−23	+9	−74	−16	−41	−17	−37	−10	−57	−41	76	326	4.29

* Loss in moisture in thirty-seconds of an ounce.

and April 10. The total number of days in which this butter was held was 155.

The shrinkage from one weighing to another on the total weight of the 12 or 13 pound prints is shown in table 1. For illustration, sample A lost $\frac{23}{32}$ of an ounce from October 28 to November 5, and increased $\frac{1}{32}$ in the next period. Then it lost $\frac{3}{32}$ from November 19 to November 26. The total shrink-

age is given in this table, as well as the average for each pound print. The computation included each weighing.

TABLE 2

Effect of working on shrinkage of butter

SAMPLE	CHURNING 1		CHURNING 2		CHURNING 3		CHURNING 4	
	Age 234 days. First weight after storage	Age 261 days. Second weight after storage	Age 228 days. First weight after storage	Age 255 days. Second weight after storage	Age 220 days. First weight after storage	Age 247 days. Second weight after storage	Age 219 days. First weight after storage	Age 246 days. Second weight after storage
Division 1. Worked sufficiently to give uniform color								
A	−9*	−10	−23	−19	−15	−16	−22	−23
B	−14	−15	−20	−17	−8	−9	−11	−11
C	−12	−17	−29	−25	−20	−22	−24	−25
D	−15	−16	−23	−21	−17	−19	−12	−12
E	−20	−20	−28	−28	−21	−23	−27	−28
F	−15	−15	−26	−25	−20	−20	−20	−21
G	−8	−8	−19	−17	−16	−17	−20	−20
H	−9	−8	−26	−25	−15	−15	−17	−18
Average..	−12.75	−13.62	−24.25	−22.12	−16.50	−17.62	−19.12	−19.75
Division 2. Worked 10 additional revolutions of Victor churn								
I	−1	−1	−11	−11	−3	−3	0	−1
J	−1	0	−6	−6	−5	−5	−2	−2
K	−2	−1	−7	−7	+2	−1	−2	−2
L	−2	−1	−6	−6	−7	−5	−3	−3
M	−1	0	−10	−11	−4	−3	−3	−3
N	−0	+1	−12	−12	−5	−5	−3	−4
O	−1	0	−6	−6	−2	−2	−7	−6
P	−1	−2	−7	−7	−4	−3	−6	−6
Average..	−1.12	−0.50	−8.12	−8.25	−3.50	−3.37	−3.25	−3.37

* Loss in moisture in thirty-seconds of an ounce.

A and A′ were wrapped in the same kind of wrapping paper. Likewise, B and B′ and the other pairs were wrapped in the same types of paper. It is noticeable that in every pair the

butter which was worked 68 revolutions of the churn did not shrink as much as did that which was worked only 50 revolutions. Some of the variations were probably caused by the moisture leaking from one print to another, for it should be remembered that this butter was packed in 50-pound boxes which contained two tiers of 25 pounds each. The prints were not always replaced in a certain position in the box after weighing. Sometimes they were in the lower tier and again they were in the upper layer. This may account for occasional plus weights. The rate of shrinkage per day from weighing to weighing throughout the whole period seemed to be approximately the same. This indicates that the butter was still a long way from constant weight, or when no more evaporation would take place.

The average loss in the 155 days at 45° to 50°F. was $\frac{6.54}{32}$ of an ounce in division 1, and $\frac{4.29}{32}$ of an ounce in division 2. The difference of $\frac{2.25}{32}$ of an ounce to each one-pound amounts to 4.4 pounds on a 1000-pound churning. When the price of butter is 45 cents a pound, the value of this difference is \$1.98.

More recent studies of shrinkage by the author indicate greater loss of moisture than is shown in table 1. The further research, which is reported in table 2, was made of the regular sweet-cream butter which is manufactured in the Cornell University creamery. It was held at 0 to +5°F. for about eight months, and was churned in a Victor churn which has two sets of rolls. When the butter was worked sufficiently to distribute the salt evenly, thus insuring a uniform color, the samples of division 1 were taken. Then the butter was worked 10 more revolutions of the churn and the samples of division 2 were obtained.

The average loss of moisture to each pound print of butter in division 1 was $\frac{18.15}{32}$ of an ounce, when first weighed after storage. When it was held 27 days longer and weighed the second time after storage the loss had increased to $\frac{18.28}{32}$ of an ounce, or 3.56 per cent of a pound. In division 2, the shrinkage was much less, for the first weight after storage showed only $\frac{3.25}{32}$ of an ounce, and it was $\frac{3.87}{32}$ of an ounce, or 0.75 per cent, 27 days later when it was weighed the second time after storage.

The shrinkage of 3.56 per cent of division 1 amounts to 35.6 pounds on a 1000-pound churning. The loss in division 2 of 0.75 per cent is 7.5 pounds in a churning of similar size. The difference is 28.1 pounds of butter, which at 45 cents a pound has a value of $12.65.

This limited study indicates that the shrinkage of butter by evaporation and leakage can be largely controlled by thoroughly incorporating the moisture in the fat in the working process.

A DEFECT OF PIMENTO CHEESE*

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INTRODUCTION

Pimento cheese is a variety of soft unripened cheese of the Neufchatel type which combines high moisture content with a high acidity. The quality of this type of cheese is largely dependent upon a clean, mildly acid flavor.

The work of this investigation was undertaken to determine the factors responsible for the sharply acid and bitter flavor which develops on standing and renders the cheese unsalable. Spore forming anaerobic bacilli like the causal organism of this defect have been studied by Esty (1), Hall (2), Reddish (3), Speakman (4), and others.

All experimental cheese was made in the laboratory where the entire manufacturing process could be controlled and uniform methods employed. The milk was received daily from the Bureau of Dairying farm and was pasteurized before being used. In making the cheese the usual factory procedure was followed throughout.

EXPERIMENTAL

In sampling the cheese for bacteriological analysis every precaution was taken to prevent contamination. The sample was removed from the center of the jar; and after weighing on a sterile paper, one gram was transferred to a mortar containing washed, ignited, sterile sand. Samples of cheese and the culture of Streptococcus lactis used in its manufacture were plated on litmus-lactose agar, litmus-dextrose agar, infusion agar, the milk

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powder agar of Ayers and Mudge (5), and a 10 per cent skimmed-milk agar. The last two media proved the most satisfactory, for they produced colonies that were larger and the acid-forming types could be more readily differentiated. Dilutions of 1:1,000,000 and 1:5,000,000 gave the most satisfactory plates. All plates were made in duplicate and incubated for three days at 26°C. Later anaerobic methods were tried.

The total titrable acidity and the volatile acidity of the cheese were determined. The total titrable acidity was determined by titration with an N/10 solution of sodium hydroxide, using phenolphthalein as an indicator. An aqueous extract of the cheese did not prove satisfactory, because, as Barthel (6) has demonstrated, the acids arising from the fermentation of the milk sugar are mainly in combination with the lime of the phosphate and the paracasein. The determinations were made in duplicate, and the figures represent an average. Cheese finely ground in a mortar was used for these titrations.

The volatile fatty acids present in the cheese were determined quantitatively by the steam distillation method of Ducleaux (7). A 100-gram sample was weighed out and placed in a large mortar with 100 cc. of distilled water. The mass was thoroughly ground with a pestle and an additional 200 cc. of distilled water placed in the receiving flask, after rinsing all particles from the mortar. Five grams of granulated zinc and 2 cc. of concentrated sulphuric acid were added to the contents of the boiling flask to bring over the volatile acids. The hydrogen liberated by the combining of the zinc and sulphuric acid kept the contents from violent agitation during the distillation process. Thirty cubic centimeters of N/10 sodium hydroxide were placed in the receiving flask and the distillation continued until 1000 cc. of the distillate was secured. This volume was titrated with N/10 HCl, using phenolphthalein as an indicator.

Streptococcus lactis was eliminated as a possible cause of the defect since data secured from three cultures over the usual six-day period showed that little change in the amounts of volatile acid took place, while no defect in flavor could be noted. This is in agreement with the results of Hammer (8), Evans (9), Leichmann (10), and Orla Jensen (11).

Bacteriological examinations of a number of samples of canned pimentoes showed that these were sterile. Both the plate method and the anaerobic technique were employed. Since the defect could be produced in cheese without the addition of pimentoes, these were eliminated as the causative factor of the sharply acid and bitter flavor.

Six lots of cheese were made in the usual manner, and daily analyses were run over a six-day period, which represents the time during which the marked sharpness and bitterness usually appeared. A study of the plates showed that 98 per cent of the organisms present during the development of the defect were acid formers of the Streptococcus lactis type. A marked increase in volatile acidity took place, ranging from 14.7 to 34.9 per cent with an average increase of 29.1 per cent. The sharply acid flavor developed two or three days before the bitterness occurred. It was apparent from these experiments that the causative organism could not be demonstrated by aerobic methods; therefore, anaerobic methods were tried.

A 1-gram shample of cheese with the sharpness and bitter flavor well developed was shaken well in 100 cc. of sterile water. Four tubes containing approximately 10 cc. each of dextrose-agar were melted in a steamer and quickly cooled to 40°C. One cubic centimeter or $\frac{1}{100}$ gram of cheese from the above 1:100 dilution was added to the first tube of dextrose-agar. In a similar manner 1 cc. was transferred from tube 1 to tube 2. This serial dilution was carried through four tubes. The work was done in duplicate, and the surface of the inoculated media of the first set of tubes was covered with melted vaseline. The second set was drawn into sections of sterile glass tubing sixteen inches long. These were filled to within an inch of each end and allowed to solidify after which all were incubated at 37.5°C. from twenty-four to forty-eight hours.

Examination at the end of twenty-four hours showed the characteristic anaerobic gas production, both in the test-tube cultures and in those in the glass tubing. This occurred through the third tube of the series which contained approximately $\frac{1}{100,000}$ gram of cheese. Growth was not apparent in the higher

TABLE 1

Cheese made from milk sterilized in the autoclave

DAY	TOTAL TITRATABLE ACIDITY	TOTAL VOLATILE ACIDITY	ROUX TUBES DILUTION SHOWING GAS	PLATE COUNT PER GRAM	MICROSCOPIC	FLAVOR
	per cent	cc. N/10 NaOH	gram			
Lot 1. Control cheese inoculated with 0.5 per cent of Streptococcus lactis alone						
First.........	1.60	17.00	Negative	1,520,000,000	Cocci	Clean, mildly acid
Second........	1.65	17.20	Negative	846,500,000	Cocci	Clean, mildly acid
Third.........	1.65	17.25	Negative	611,500,000	Cocci	Clean, mildly acid
Fourth........	1.75	17.50	Negative	329,500,000	Cocci	Clean, mildly acid
Fifth.........	1.75	17.60	Negative	342,500,000	Cocci	Clean, mildly acid
Sixth.........	1.78	17.85	Negative	386,500,000	Cocci	Clean, mildly acid
Lot 2. Cheese inoculated with 0.5 per cent Streptococcus lactis and anaerobic culture of "shocked" spores						
First.........	1.78	17.20	Negative	218,500,000	Cocci	Slightly sour
Second........	1.85	17.40	1/10,000	247,500,000	Cocci	Increased sourness
Third.........	1.88	17.65	1/10,000	184,000,000	Cocci few medium rods	Sharply sour
Fourth........	1.90	19.45	1/10,000	135,500,000	Cocci few medium rods	Very sharply sour marked slight bitterness
Fifth.........	1.95	20.10	1/1,000,000	71,000,000	Cocci rods predominate	Very sharply sour, very bitter
Sixth.........	1.97	20.50	1/1,000,000	52,500,000	Cocci	Very sharply sour, very bitter

Lot 3. Cheese inoculated with Anaerobic Culture of Shocked Spores Alone

First...........	1.65	17.50	1/10,000	18,000	Medium sized rods	Very sour
Second.........	1.65	17.40	1/10,000	5,200	Medium-sized rods	Increased sourness
Third...........	1.72	17.80	1/10,000	27,000	Medium-sized rods predominating few small cocci	Extreme acid flavor
Fourth.........	1.80	18.90	1/1,000,000	3/800	Medium-sized rods	Slight bitterness
Fifth...........	1.82	19.20	1/1,000,000		Medium-sized rods	Very bitter
Sixth...........	1.84	19.20	1/1,000,000	7,000	Medium-sized rods	Very sharp and bitter

dilutions after 48 hours incubation. The presence of this anaerobe was also demonstrated in three market samples having the characteristic defect well developed. The increase in the volatile acidity coincident with the marked changes in flavor occurring in these market samples was in accordance with the results secured on the six samples of the laboratory-made cheese. This was considered sufficient proof that the cause of the defect occurring in the laboratory cheese was the same as that of the commercial product. The anaerobe was isolated and found to be similar to Clostridium butyricum (B. amylobacter) in its characteristics.

The sharp bitterness which occurred in the cheese with large numbers of the spore-forming anaerobic bacilli present made it seem likely that the cause of the defect was this anaerobe. If the anaerobic culture isolated from the laboratory-made cheese and the commercial samples was the causative factor in the production of the sharp bitter flavor in these cheeses, it should be possible to reproduce these changes in specially controlled cheese by inoculation with this anaerobic culture.

Since investigators working with the butyric type of fermentation caused by B. amylobacter have shown that a more vigorous fermentation occurred when the spores of the organism had been "shocked" or subjected to high temperatures, this process was also tried in this experiment.

The milk used for the experiments in table 1 was sterilized in the autoclave and divided into three lots. Each of the three lots was inoculated with 0.5 per cent of a twenty-four-hour culture of Streptococcus lactis. To lot 1, which was the control, nothing was added. Lot 2 was inoculated with 0.5 cc. of the "shocked" spores of anaerobic organism. This culture of "shocked" spores was prepared by heating old cultures of the anaerobe to 85°C. for fifteen minutes. Lot 3 was inoculated with 0.5 cc. of the unheated (normal) spore culture of the anaerobe. Every precaution was taken to avoid contamination throughout the manufacture of the cheese. A microscopic examination of each lot was made daily using the Breed technique.

The data in lot 1, which is the control cheese, showed that the

total titrable acidity and volatile acidity were only slightly increased. The flavor remained clean and mildly acid throughout the entire period. The per cent of increase in volatile acidity was 4.7. This, together with the negative Roux tubes, the plates, and the microscopic examination, is believed to be ample evidence that the control cheese had not become contaminated.

The data in lot 2, which contains the shocked spores of the anaerobe, showed that a marked increase in volatile acidity took place. The increase was 16 per cent as compared with an increase of only 8.8 per cent in lot 3, where the unheated spore culture was used. The marked increase in volatile acidity accompanied by a more rapid development of the bitter flavor in the cheese having both Streptococcus lactis and the spore culture of the anaerobe is considered strong evidence of an associative action of these two organisms in the development of the defect. The bitterness was noticeable early on the fourth day, and the cheese became very bitter by the end of the fifth day. The anaerobe was demonstrated in the $\frac{1}{1,000,000}$ gram dilution on the fifth day. The defect described above appeared more rapidly and was more pronounced in the experimental cheese having the "shocked" spore culture of the anaerobe.

The anaerobic organism used in these experiments is a gram positive motile bacillus of medium size. Colonies in deep dextrose-agar tubes are compact with a very definite darker center. Litmus milk is rapidly reduced and coagulated, followed by abundant gas production. The curd is fragmented and the butyric-acid odor is very noticeable. Indol is not formed. Nitrates are reduced to nitrites. The organism appears similar to Clostridium butyricum (Bacillus amylobacter) according to Bergey's classification.

SUMMARY

A brief summary of the results of this work is given below:

1. Streptococcus lactis was proved not responsible for the sharply acid and bitter flavor.

2. The pimentos were not responsible for the defect.

3. Aerobic methods failed to disclose the causal organism.

4. Nine samples of sharply acid and bitter cheese were found to contain large numbers of spore-forming, gram positive, motile anaerobic bacillus which resembles Clostridium butyricum.

5. The increase in sharply acid and bitter flavor was always accompanied by a corresponding increase in volatile acidity.

6. The characteristic defect was produced experimentally by the use of a pure culture of the anaerobic bacillus.

7. "Shocked" spores of the anaerobic bacillus produced a more active fermentation than the unheated spores.

8. Pasteurization of raw milk is equivalent to this method of shocking the spores.

9. The associative action of the Streptococcus lactis and the anaerobic bacillus proved more effective than the anaerobe alone.

CONCLUSION

The sharply acid and bitter flavor, commonly found in old pimento cheese of the Neufchatel type, is due to the action of an organism resembling Clostridium butyricum, which may act in association with the Streptococcus lactis of the starter.

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ELIMINATING THE TOXICITY OF COTTONSEED MEAL*

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INTRODUCTION

Studies to determine the cause of deleterious effect arising from the feeding of cottonseed meal to certain types of live-stock and other animals, have been carried out to such an extent and such evidence brought to light on the subject, that we feel quite safe in assuming the trouble to lie in the toxic properties of a substance isolated from the cotton seed and called gossypol (1, 2). Such an assumption is not without criticism however, and it does not necessarily follow that wherever injurious effects are encountered with the use of cottonseed meal as a feed, the only cause is due to gossypol. Yet, the principal cause of the injury, when balanced rations are being used, is no doubt due to this substance.

Other substances which have been suggested as productive of the symptoms of toxemia and malnutrition have been investigated and the results summarized by Macy in this JOURNAL (3). Among such substances are the nitrogenous compounds Cholin and Betain, the high protein content, the fiber, parasitic organisms, decomposition products, oil content, and compounds of phosphorus. Other investigators have suggested that the trouble is due to a deficient diet (4, 5, 6, 7). Most of our later evidence, however, is not in favor of these theories, and the results of recent investigations carried on to a large extent by Southern Experiment Stations tend to prove that gossypol is the main cause of the trouble. Unpublished results at this station also point in that direction.

[1] These animals were obtained without charge from V. G. Heller.

We are particularly interested in cottonseed meal as a foodstuff for several reasons and feel that too much stress cannot be laid on the needs for investigation of its use in feeding different kinds of farm animals. Henry and Morrison (8) state, "Since the meal is a heavy, highly nitrogenous feed, and is poisonous to fattening cattle when fed in excess, the determination of the allowance to be fed for the best results is of great importance." These authors go on to say, "It is to be hoped that in time methods may be developed of treating cottonseed meal so that it will be safe for all classes of animals." Much of the work at this station has been conducted along this last line of investigation and we have met with very favorable results.

Not only is cottonseed meal highly nitrogenous but it contains protein of such excellent quality that when the meal furnishes the sole source of protein in a diet for albino rats, growth and reproduction are normal (5). This is a fact not true for many feeds. The work of Nevens (9, 10) on the amino acid content and nutritional value of cotton seed protein has contributed much to our knowledge of this important factor. Nevens found that when cottonseed meal furnished the sole source of protein in a ration containing 10 per cent crude protein, the utilization of the protein for the growth of albino rats was 66 per cent. In a similar way he found the utilization of the protein of alfalfa to be 62 per cent and that of corn 49 per cent. His investigation was carried out by first studying the endogenous metabolism of the experimental animals during a metabolism period in which a nitrogen-free ration was fed and then followed by six such metabolism periods in which the proteins of these feeds were compared.

The digestibility of the protein from cotton seed as found by other investigators (11, 12, 13, 14) is not in close agreement but probably lies between 80 and 85 per cent. Rather (13) finds this value to the 78.6 per cent which is about the same as that for the protein of legumes, but less than the value as found for cereals and meat. Since the isolated cottonseed globulin, even when fed in small quantities (15) has been shown to be able to support normal growth, Jones and Waterman (14) believe that the isolated protein is more digestible than the protein in combination with

other substances in cotton seed and conclude that the lower digestibility is due in part at least to the presence of gossypol. The digestibility of cottonseed meal in which the gossypol has been removed or destroyed would be well worth studying.

As a result of the foregoing investigations together with many practical experiments on the feeding value of cottonseed meal it seems desirable that we learn more concerning its use in large quantities without deleterious results to the animals fed. The most important facts with which the feeder is concerned are, that the meal contains a high per cent of protein which is of excellent quality and quite digestible, that the price of the meal is comparatively low, and that the manurial value is exceptionally high. Its toxicity, however, is a drawback to its extensive use.

Macy (3) (1921) states, "Many attempts have been made to render cottonseed meal non-toxic, but none have been successful for all species alike." Among the attempts are those of Edgerton and Morris (16) who subjected the meal to various treatments such as digestion in hot water, 95 per cent alcohol, and ether; fermentation, heating with hot air, over oil, and autoclaving the meal in both a dry and wet condition. All their experimental animals (guinea pigs) either lost weight or died. Withers and Ray (17) were able to reduce the toxicity of the meal by boiling it with alcoholic sodium hydroxide for two hours. Later, iron salts were suggested as an antidote and Withers and Carruth (18) found that when iron and copper salts were fed with the meal, pigs made better gains. Osborne and Mendel (15) who fed cotton seeds previously steamed for various lengths of time and then dried were unable to draw any definite conclusions to the benefit of such treatment. The latest work has been done by Dowell and Menaul (19) who show that autoclaving the wet meal at 15 pounds pressure for twenty minutes destroys the poison peculiar to it and makes a safe feed for young pigs.

Recent investigations have been carried out principally along two lines. One of these is based on the theory that the injury is due to a deficient diet, while the other has for its foundation, the isolation of the toxic gossypol. Many feeders believe that the conditions under which animals are kept when being fed on cot-

tonseed meal have much to do with the results of such feeding and when the animals are on pasture they are able to consume much larger amounts of meal without bad effects. Also that silage makes an exceptionally good supplement. These things might well be true, and it is barely possible that the supplement used may be of such a nature that it inhibits the effect of gossypol or possibly combines with it to form an insoluble compound or destroys it altogether. Such possibilities must be kept in mind when considering the real cause of cotton seed injury. It is more probable that certain supplements have a specific effect on the gossypol than that they make up for a deficiency which would otherwise result in injury to the growth of the animal. Iron salts have been proven to be beneficial to the health of pigs receiving cottonseed meal and since we know that gossypol forms an insoluble compound with iron we might well believe that their corrective effect is due in part to their combination with gossypol in the meal. What effect silage and the compounds contained therein might have upon gossypol is well worth investigating. The value of pasture and source of roughage fed with the meal is subject to diverse opinions.

The dairy industry is particularly interested in cottonseed meal since it constitutes one of the principal sources of protein and any condition which may increase the food value of this substance will be of direct interest to it. Moore (20) reports that for cows as much as 5 pounds per head daily is injurious if fed for any length of time and that it causes difficult breathing and inflammation of the udder, while for calves it has been reported that as much as $\frac{1}{4}$ to $\frac{1}{2}$ pound per head daily is fatal (21). Calves and swine are especially susceptible to the effect of cottonseed meal feeding while sheep, as found by Dowell and Menaul (19) show no signs of ill effects even after being fed 1 pound of the meal daily for a period of ninety days. The dairy herd at this station is receiving a ration which contains about 10 per cent cottonseed meal and when this amount is increased, silage is usually fed as a supplement.

Recent reports at the North Carolina Station (22) show that calcium carbonate, butter fat, cod liver oil, yeast and other sup-

plements have a corrective effect when excessive quantities of cottonseed meal are fed to cows and heifers. Our own results, which were obtained by feeding albino rats a ration in which calcium carbonate, butter fat, and yeast supplemented the cotton seed, are not in harmony with theirs and are reported later in this paper. However, the methods of conducting the two experiments have probably been so diverse that the two results are hardly comparable.

The following investigations were undertaken for two principal reasons. First, to continue to work of Dowell and Menaul on the effect of autoclaving upon the toxicity of cottonseed meal and, second, to find out what effect certain supplements might have when large amounts of cotton seeds are fed. In doing this young pigs and albino rats were used as experimental animals. Although albino rats are not as susceptible to the effects of cottonseed meal as are guinea pigs, we were better equipped to care for rats and found that they responded quite readily to the effects of the cotton seed diets used. The results obtained by feeding young pigs are probably the same as might be expected in feeding calves similar rations and thus allow for somewhat general conclusions. According to Henry and Morrison (8) (1923), "No uniformly successful method of feeding cottonseed meal to swine has yet been found."

EXPERIMENT A

Twelve young pigs were divided into 4 lots of 3 each, using one lot for a control, and the other three for studying the effect of feeding commercial cold pressed cottonseed meal as it is brought on the market and the same meal autoclaved and steamed. All the pigs were innoculated against cholera and scrubbed with cresote to rid them of lice before the actual experiment began. One of the pigs in the pen receiving the autoclaved meal became sick shortly after the experiment began and was replaced by a pig from the control pen. The death of the animal, which occurred sometime later could not have been due to cotton seed injury since the feeding had only progressed a few days previous to the animal's sickness. The pigs were weighed at the

beginning and at the end of the experiment which lasted seventy-five days.

The autoclaved meal was prepared by dampening the commercial meal and autoclaving it at 20 pounds pressure for one hour. The time of autoclaving was somewhat longer than that used by other investigators (19) but by so prolonging the time the meal became more thoroughly cooked. The thoroughness of cooking was determined by the odor and color of the meal. The autoclaved meal when removed from the autoclave has a brown color throughout and a pleasant cooked odor. When this cooking treatment is not complete the center of the mass becomes only slightly brown and that portion of the meal retains its toxic properties. The steamed meal was prepared by passing steam directly into the meal contained in a large tin container with a false bottom. Holes were punched in the bottom of the container and it was packed with excelsior around the sides into a half barrel. A tap was fitted in the bottom of the barrel to allow the excess water formed by the condensed steam, to escape. The temperature was kept close to 100°C. Much of the steam condensed during the process which lasted for about one hour and the meal, although very wet when removed, was thoroughly cooked throughout.

The rations for the pigs receiving the cottonseed meal were made up of corn chop 3.3 pounds, cottonseed meal 1.2 pounds, and alfalfa. During the last month of feeding part of the corn chop was replaced with wheat bran but during the entire experiment the amount of cottonseed meal fed daily constituted approximately 1.3 per cent of the body weight of the animal. The amount of feed was thus increased proportionally as the pigs grew.

The control pen contained only two pigs receiving a ration composed of corn chops 3 pounds, tankage 0.5 pound, and alfalfa. These pigs did not appear as strong or eat as well as the others.

The pigs receiving the steamed and autoclaved meal were in the best condition throughout the experiment and always ate all of their feed. The pigs receiving the untreated meal ate sparingly, picking out the corn as much as possible from the meal, and often leaving considerable feed. By occasionally making a

slop of the uneaten residue with the next day's feed this condition was remedied.

The results of the experiment are shown in table 1 which gives the weights of the pigs in pounds, the form in which the cottonseed meal was fed to each lot, and the gains made.

TABLE 1

Growth of pigs on cottonseed meal rations

NUMBER OF PIG	ORIGINAL WEIGHT	FINAL WEIGHT	
Lot I			
1	25	34	Untreated cottonseed meal
2	25	35	Total gain: 40 pounds
3	39	60	Gain per pig: 13 pounds
Total.......	89	129	
Lot II			
1	33	53	Autoclaved cottonseed meal
2	23.5	51	Total gain: 66 pounds
3	37.5	56	Gain per pig: 22 pounds
Total......	94	160	
Lot III			
1	26.5	40	Steamed cottonseed meal
2	39	74	Total gain: 77 pounds
3	31.5	60	Gain per pig: 26 pounds
Total.......	97	174	
Lot IV			
1	20	33	No cottonseed meal
2	18	35	Total gain: 30 pounds
Total.......	38	68	Gain per pig: 15 pounds

Although the pigs receiving the steamed cottonseed meal made the largest gains, none of the pigs increased in weight as rapidly as might be expected. This was probably due to the amount of bulk in the ration since cold pressed meal contains a large amount of crude fiber and does not therefore make as suitable a feed for

pigs as the hot pressed meal. The pigs which were in the poorest condition were used in the control pen in order that those receiving the cotton seed meal rations might be of about equal size and weight. The difference in the gains made by the control pigs and those receiving the cottonseed meal, therefore, cannot be used for comparing the two rations and further work would need to be done to obtain the feeding value of cooked cottonseed meal as compared to other concentrates.

<center>EXPERIMENT B</center>

In order to continue this work with animals which require a smaller amount of food and permit the use of purified forms, albino rats were used for experimental animals.[1] It had previously been found (15) that rats receiving in their diet cottonseed kernels which had been steamed from one to six hours grew better than those receiving the untreated kernels. However, even after steaming for one hour, the kernels proved to be still toxic and the rats thus fed lost weight. Furthermore, the rats which were fed kernels steamed for six hours made smaller gains than those fed the kernels steamed for two and four hours. It would seem from this that one hour of steaming was not sufficiently long to bring about the changes in the meal desirable to produce a safe feed, whereas six hours of steaming were too long. If the improvement of the kernels as produced by steaming is due to the destruction of the gossypol, then the above results would be expected to be more uniform.

Keeping in mind the possibility of feeding cottonseed products in a well supplemented diet without producing injurious effects, the rations shown in table 2 were made up and fed.

The cotton seeds which were obtained from the agronomy department were delinted with sulphuric acid and fed in the ration without removing the hulls. When used in rations II and III they were soaked about four hours previous to autoclaving. Such treatment softened the hulls and allowed the seeds to soak up considerable water such that when they were subsequently autoclaved, the steam was able to penetrate the kernel. Although the seeds were not autoclaved in such large quantities

as was the meal in previous work, yet this method of destroying the gossypol appeared to be more thorough and there was practically no loss of oil or other constituents. The cotton seeds used in ration IV were first extracted with ethyl ether which removes both the gossypol and oil. Commercial cottonseed oil was then added to the ration. Distilled water containing a trace of KI was supplied for drinking.

The result of this feeding which extended over a period of nine weeks is shown in table 3.

TABLE 2
Cotton seed rations for rats

	I	II	III	IV
	per cent	per cent	per cent	per cent
Wheat..............................	50	50	50	50
Cottonseeds, untreated...............	35			
Cotton seeds, autoclaved at 20 pounds for one hour............................		35		
Cotton seeds, autoclaved at 20 pounds for two hours...........................			35	
Cotton seeds, extracted................				25
Butter...............................	6	6	6	6
Agar agar............................	3	3	3	3
Yeast	5	5	5	5
CaCO₃...............................	1	1	1	1
Cotton seed oil.......................				10

All the animals receiving the uncooked cotton seeds were in poor conditions during the latter part of the experiment and died within a short time after the experiment ended. The other animals made about normal gains and grew to be healthy strong rats producing normal young. Death in all four cases was prolonged, the rats having made no consistent gains after the first three weeks but usually lost weight thereafter. Although acute cases of poisoning have been noted, it usually takes this slower form which makes recovery possible providing the symptoms are recognized and changes made in the diet.

From these results it is clear that autoclaving the cotton seeds as described is beneficial and allows them to be used in large

quantities in a ration which is supplemented with the necessary minerals and vitamines to produce normal growth. Autoclaving for two hours does not seem to have any advantage over the one hour treatment.

Since some investigators claim that the feeding of cottonseed meal produces no injurious effects upon albino rats and that the ether extracted meal has no advantage over the ordinary meal (5), it seemed desirable to find out if the steamed meal has any advantage over the ordinary meal. When working with the meal, other factors enter in which cannot be disregarded as is done when feeding the cotton seeds. The most important factor

TABLE 3

Growth of rats on cotton seed rations

FORM OF COTTONSEED	UNTREATED				AUTOCLAVED ONE HOUR				AUTOCLAVED TWO HOURS			EXTRACTED		
	Ration I				Ration II				Ration III			Ration IV		
	Rat 1 ♂	Rat 2 ♀	Rat 3 ♂	Rat 4 ♀	Rat 5 ♂	Rat 6 ♂	Rat 7 ♂	Rat 8 ♂	Rat 9 ♂	Rat 10 ♀	Rat 11 ♀	Rat 12 ♂	Rat 13 ♂	Rat 14 ♂
Original weight, grams..........	71	66	88	82	99	86	85	92	106	85	92	92	92	95
Final weight, grams..........	79	67	85	67	173	147	137	146	205	135	126	140	168	160
Gain or loss, grams........	+8	+1	−3	−15	+74	+61	+52	+54	+99	+50	+34	+48	+76	+65

is the destruction of the gossypol or its conversion into a less soluble compound during the heating process previous to pressing out the oil. At the oil mill, the decorticated seeds before the oil is pressed out, are heated (for about thirty minutes at a temperature of about 100°C.), in steam-jacketed drums but are not subjected to the direct action of the steam. The oil is then pressed out of this hot mass, taking with it much of the gossypol. The remaining cake is broken up or ground and sold as "hot pressed" meal. During this preliminary heating some of the gossypol is undoubtedly destroyed but the amount must be very small and will vary with the process. A considerable amount of

the gossypol, however, may be altered such that it is less soluble in the oil and therefore retained in the meal. If this less soluble form is as toxic as the original form then we would expect to find the resulting meal almost as toxic as the seeds. Since this is not true, it is then supposed that the gossypol is rendered less toxic at the same time that it is converted into a less soluble compound and the completeness of this reaction will depend upon the conditions which prevail during the preparation of the meal. Some manufacturers heat the seeds for a much longer time than thirty minutes and in many cases the pressure of the steam surrounding the drums ranges from 30 pounds upward. Since the moisture content of the seeds is also quite variable and seems to influence the destruction of gossypol, methods by which the meal is produced will vary from one mill to another. Due to these conditions, no two samples of cottonseed meal as bought may be alike in their toxicity and it is due to this condition that cotton seed meal is sometimes reported as nontoxic to rats.

To determine the advantage of steaming the meal before feeding to rats, the rations given in table 4 were made up and fed to three groups of rats for a period of seven weeks.

The cottonseed meal used was purchased on the open market and when used in ration XVII was steamed as previously described in experiment A.

The rates of growth are shown in table 5.

As in the case where autoclaved cotton seeds were used, the steamed meal has a marked advantage over the ordinary meal and may be fed in much larger amounts. The use of only 25 per cent cottonseed meal did not appear to be toxic to the rats during the seven weeks of feeding and it is possible that, that amount of the meal can be fed to rats for a longer period of time without retarding their growth. However, by increasing this amount 10 per cent, slow growth and poor condition are noticeable in a short time.

Such a condition as this often arises in stock feeding when there is a certain limit beyond which the amount of cottonseed meal in the ration cannot be increased with safety. This difficulty

may be overcome without changing the other constituents of the ration by steaming the meal previous to its use.

Further proof of the advisability of such treatment and the increased nutritive value of the cotton seed is shown in the growth made by a group of rats fed solely on autoclaved cotton seeds and

TABLE 4

Cottonseed meal rations for rats

	XVII	XVIII	XIX
	per cent	per cent	per cent
Wheat..................................	58	58	69
Cottonseed meal steamed one hour......	35		
Cottonseed meal untreated.............		35	25
Butterfat..............................	5	5	5
CaCO₃.................................	1	1	1
NaCl..................................	1	1	1

TABLE 5

Growth of rats on cottonseed meal rations

FORM OF COTTONSEED MEAL	STEAMED ONE HOUR				UNTREATED				UN-TREATED	
	Ration XVII				Ration XVIII				Ration XIX	
	Rat 43 ♀	Rat 44 ♂	Rat 45 ♀	Rat 46 ♂	Rat 47 ♂	Rat 48 ♂	Rat 49 ♀	Rat 50 ♀	Rat 51 ♀	Rat 52 ♀
Original weight, grams.........	28	28	29	35	40	36	28	28	28	28
Final weight, grams............	102	121	115	158	80	80	27*	31†	100	110
Gain or loss, grams.............	+74	+93	+86	+123	+40	+44	−1	+3	+72	+82

* Rat 49 ♀ died after five weeks feeding.

† Rat 50 ♀ died after three weeks feeding.

a 2 per cent salt mixture composed of equal parts of NaCl and CaCO₃. Their growth is shown in table 6.

Rats 29 ♂ and 30 ♂ both made small gains until the time of their death which occurred before the experiment ended. Rats 27 ♀ and 28 ♂ gained slowly during the first eleven weeks of feeding and then declined in weight until June 26 when 5 per cent butter fat was incorporated in the ration. From then until the

experiment ended they made fair gains. Similarly rat 30A ♂ which replaced rat 30 ♂ gained during the first four weeks and then lost weight until the butter fat was added. The slow growth of all the animals and the death of two of them were due in a large degree to the lack of the fat soluble vitamine. The results are quite satisfying, however, when the unbalanced nature of the ration and the toxicity of the raw seeds are considered.

TABLE 6

Growth of rats on cotton seeds and salt mixture

	WEIGHT				
	Rat 27 ♀	Rat 28 ♂	Rat 29 ♂	Rat 30 ♂	Rat 30A ♂
	grams	grams	grams	grams	grams
February 21.........	49	57	57	78	
April 16............	71	71	75	85 (Died)	
May 20.............	75	85	92 (Died)		78
June 26.............	62	71	(Butter fat added)		78
August 1...........	92	113			100
Gain.............	+43	+56	+35	+7	+22

These results lead us to believe that the untreated cottonseed meal is quite toxic when fed in too large quantities or when fed to young animals. Yet by proper treatment, it may be prepared so that it becomes an excellent source of protein for supplementing protein deficient rations.

CONCLUSIONS

1. Cottonseed meal can be made a safe feed for swine, and probably other livestock as well, by autoclaving or steaming the meal until it is thoroughly cooked.

2. Such treatment renders the meal more palatable and in so doing raises its value as a feed.

3. A deficiency in the ration cannot account for the difference in the gains made by pigs receiving the untreated cottonseed meal and those receiving the cooked meal.

4. Supplements such as $CaCO_3$, yeast, and butterfat when

used to produce a balanced diet do not overcome the toxic effect of either the cotton seeds or the meal when fed to albino rats.

5. Animals may make small gains extending over a considerable length of time on rations containing a limited amount of cotton-seed meal—above that limit they lose weight and usually die if the feeding is continued.

6. Cooking the meal increases the amount which may be safely fed over any period of time.

In conclusion the author expresses his thanks to P. L. Menaul for many helpful suggestions during the progress of this work.

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ENERGY REQUIREMENTS OF DAIRY COWS

A REPLY TO ARTICLES BY E. B. MEIGS AND H. T. CONVERSE

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In this JOURNAL for May, 1925, appeared an article by E. B. Meigs and H. T. Converse, on the Quantities of Nutritive Energy Necessary to Maintain Dairy Cows in Nutritive Equilibrium, in which the authors presented a critical comparison of the feeding standards for maintenance of dairy cows, as proposed by Henry and Morrison, by Eckles, and by Armsby; and also a comparison of digestible nutrients and net energy, as measures of the nutritive value of feeds; and in the November, 1925, number of this JOURNAL appeared a second article, by E. B. Meigs, on the energy requirements of dairy cows, under the title, Is Net Energy or Metabolizable Energy the More Useful Index for Practical Purposes.

Referring first to the second article, this consists of a criticism of the respiration experiments of Kellner and Armsby—the two preëminent scholars in the field of energy metabolism of farm animals. In discussing Armsby's work, however, Dr. Meigs bases his criticisms and conclusions mainly on items selected from his earlier, pioneer studies which are no longer fairly representative of the work of this Institute. All of this older work has been recalculated and revised in the light of recent advance in methods and understanding.

Since Armsby's death this Institute continues its program of measurement of the nutritive values of feeds, in terms of energy, adhering to the fundamental conceptions advanced by Armsby, and employing the most elaborate methods of research in use anywhere in the world. It stands committed to no particular figures for nutritive value, or nutritive requirements. The published figures for nutritive values and requirements may have to be revised again and again before the best possible results are

finally obtained; but we are committed to the principle of determining the nutritive value of feeds, in terms of energy, by the subtraction from their gross energy (fuel value) the total of the loses and expenses of feed utilization (likewise, of course, in terms of energy), the results constituting their net energy values.

During recent years—even during recent months—we have made important progress in methods of work, and in spite of an apparently satisfactory grasp of our subject it is not unlikely that we have further important progress in methods still to make. We do not claim, therefore, to have obtained final results, but we do claim that we are investigating the energy value of animal feeds in the most scientific method thus far devised, and we invite the interest and support of those whom we are seeking to serve, with perfect confidence in the successful outcome of these studies of net energy values and requirements.

As for the practical significance of the results, with the obviously necessary allowances for the differences between the standard conditions of scientific study and the varying conditions of scientifically uncontrolled practice, such as are necessary in the application of all exact knowledge, net energy values certainly do apply in practical animal production.

Referring now to the first article—in the experiment by Dr. Meigs upon which this article is based there appear not to have been even so much as feed analyses, to say nothing of digestion trials—in other words, all of his data for nutritive values and requirements appear to have been computed, or assumed.   There are, therefore, in reality, no experimental results to discuss except weights of cows and weights of feeds, which, of course, unsupported by other data, are not competent in relation to fundamental nutritive considerations.

We must insist, therefore, that the conclusions, in terms of digestible nutrients, and net energy, are not in any way warranted by the data presented.

On page 178 of the JOURNAL for May, 1925, Meigs and Converse say, "Armsby bases his standard on an experiment of Kellner in which the complete energy balance was determined for a

milking cow (1, p. 493–500, 511–516)." (These page numbers refer to Armsby's book The Nutrition of Farm Animals.[1])

In a footnote on the same page Meigs and Converse say, "Although Armsby considers the results obtained by Kellner with three cows, he bases his standard on the performance of the most economical of the three, rather than on the average."

This gives the impression, clearly, that Armsby considered no experiments other than Kellner's, and that he based his standard upon absurdly slight and improperly selected evidence.

Armsby explains with great particularity, in the pages cited by Meigs and Converse, the evidence and considerations upon which his standard for milk production was based.

This standard was derived (1) by allowing for maintenance the same amount of energy as for other cattle, and then (2) by computing from Haecker's analyses of milk (par. 604) the equivalents of net energy for fattening, plus 5 per cent, for the production of milk of the different grades as to fat content, in the light of the following assumptions (a) that digestible carbohydrate and protein in the feed are converted into the corresponding compounds of milk without loss, and (b) that the expenditure of energy in the production of milk fat from carbohydrates is the same as that observed by Kellner for the production of body fat (par. 593).

The evidence which Armsby considered and which he discussed as contributing to the establishment of this standard is, in the main, as indicated below:

1. The standard for maintenance was adopted in consideration of results of Armsby and Fries, Kellner, Haecker, Evvard, and Eckles (par. 381).

2. Conclusions as to utilization of feed protein in the synthesis of milk protein were based on results of experiments by Jordan, Hayward, Kellner, Hart and Humphrey, Haecker, and Royal Vet. and Agr. High School, Laboratory for Agricultural Research (Copenhagen) (par. 586).

3. In relation to the requirement of protein in the ration

[1] Armsby, H. P. 1917 The Nutrition of Farm Animals, 743 p., illus., New York.

Armsby also considered the effects of the plane of protein intake on the production of milk, as indicated by the results and conclusions of Jordan, and Morgen (par. 599), Wolff and Lehmann, Woll, and Phelps (par. 601), and Haecker (par. 602).

4. Conclusions relating to the utilization of energy for milk production were based on three complete energy balances by Kellner (par. 589), four partial balances by Jordan (par. 590), and also a consideration of Haecker's nine years' results of feed, computed digestible nutrients, live weight, and milk production, with the Minnesota Station herd of cows (par. 590).

5. The addition of 5 per cent to the equivalent energy for fattening, for each grade of milk, rests upon Eckles's finding (par. 722) that with well-fed cows the digestion coefficients were, on the average, 5 per cent lower than those which have been used in computing net energy values.

In spite of the very careful study upon which this standard was based Armsby regarded it as only tentative, as is shown by his own comment, as follows:

By this device of reducing the total energy content of the milk to the equivalent amount of net energy for fattening, it appears possible to utilize the net energy values of feeds obtained by Kellner and others in maintenance or fattening experiments as a basis for computing rations for milk. Such a method is, of course, provisional, and the basis for it at present is somewhat slender, but it seems the best one now available.

Kellner's energy balances with three cows, were used by Armsby, along with much other evidence, as indicating the higher utilization of energy for milk production than for fattening, and as confirmatory of the hypothesis in accord with which he explains the higher net energy values of feeds for milk production than for fattening.

These energy balances of Kellner, therefore, were contributory only in an indirect and general manner, and do not enter at all into the computation of Armsby's standard.

The energy requirements for milk production, according to Armsby's standard, are expressed not in terms of percentages of

utilization of metabolizable energy but in terms of net energy equivalents for fattening; and this standard calls for much more net energy (for fattening) per 100 calories of milk energy than the amounts indicated by the results of Kellner's experiment. This is clearly shown by a comparison of the data in tables 1 and 2.

TABLE 1

Energy equivalents for fattening per 100 calories of milk energy as indicated by the results of Kellner's experiments

	NET ENERGY FOR FATTENING PER 100 CALORIES OF METABOLIZABLE ENERGY	MILK ENERGY PER 100 CALORIES OF METABOLIZABLE ENERGY	EQUIVALENTS OF NET ENERGY FOR FATTENING PER 100 CALORIES OF MILK ENERGY
	calories	*calories*	*calories*
Cow A..........................	48.02	68.41	70.2
Cow C..........................	46.35	72.80	63.7
Cow E..........................	43.81	66.91	65.5

TABLE 2

Energy equivalents for fattening for different grades of milk according to Armsby's standard

GRADE OF MILK	ENERGY CONTENT PER POUND	EQUIVALENT NET ENERGY FOR FATTENING PER POUND	EQUIVALENTS OF NET ENERGY FOR FATTENING PER 100 CALORIES OF MILK ENERGY
per cent fat	*calories*	*calories*	*calories*
3.0	278	214	77.0
4.0	336	265	78.9
5.0	390	315	80.8
6.0	440	361	82.1
7.0	492	408	82.9

All figures except the last column in both tables are quoted from pages (494, 495, 511 and 513) of Armsby's book which are included in the citation by Dr. Meigs.

The results obtained with Kellner's most efficient cow (cow C) show a net energy equivalent for fattening as low as 63.7 calories per 100 calories of milk energy, while according to Armsby's standard the net energy equivalents for fattening, per 100 calories of milk energy, range from 77.0 calories to 82.9 calories for the different grades of milk, as indicated in table 2.

Thus, in the light of Armsby's own explanation of how he arrived at his conclusions the following statements of Meigs and Converse are clearly erroneous. "Armsby bases his standard on an experiment of Kellner in which the complete energy balance was determined for a milk cow. . . . . " "Although Armsby considers the results obtained by Kellner with three cows, he bases his standard on the performance of the most economical of the three, rather than on the average."

VITAMIN STUDIES

XIII. VITAMIN B IN EVAPORATED MILKS MADE BY VACUUM AND AERATION PROCESSES*

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The investigational work described in this paper is the first of a series of coöperative studies being made at the Agricultural Experiment Station of the Pennsylvania State College on evaporated milks. This work is a continuation of a larger project concerning the various factors influencing the vitamin content of milk and dairy products. Former work has dealt with dietary factors (1) (2), heat and pasteurization (3), oxidation (3) (4), and commercial drying by the spray process (5).

In the past practically all of the data available, relative to the vitamin content of evaporated milks, have been obtained on samples the past history of which has not been known. Furthermore, few attempts have been made to compare the evaporated milks with the raw milks from which they were made. As a result few authoritative data have been obtained concerning the influence of commercial evaporation processes on the stability of the various vitamins.

The present studies were undertaken with the view of preparing evaporated milks under conditions approximating, as closely as possible, the conditions used in commercial practice and comparing these products with the original milk from which they were made, to eliminate the effect of any fluctuation in vitamin content that may take place in raw milk obtained from various sources and at different seasons of the year.

THE EVAPORATED MILKS

The raw milk, from which the evaporated milks were made, was obtained, daily, from the college herd and was fed to the experimental animals within a few hours after the morning milking, although a portion of the raw milk represented the milking of the previous evening.

Two types of evaporated milk were prepared, the first being manufactured by the vacuum process in a Rogers 18-inch copper vacuum pan and the other by the aeration method, passing a current of air through the hot milk. The object in using the methods just described was to determine, if possible, the susceptibility of vitamins to oxidation during the evaporation process. We also desired to study the effect of subsequent sterilization, for sterilization plays an important rôle in the manufacture of commercial evaporated milks. At this point it is well to emphasize the fact that the writers do not claim to have duplicated commercial methods of manufacture but the attempt has been made to approximate commercial conditions as closely as possible.

EVAPORATED MILK MADE BY THE VACUUM PROCESS

While the raw milk was fed daily to the experimental animals, it was impossible to manufacture daily "batches" of evaporated milks. As a result the evaporated milks used in this study were manufactured every three weeks.

Prior to the evaporation process, milks from the evening and morning milking were mixed thoroughly and samples were taken for analysis. These samples were analyzed for titratable acidity, total solids and fat. The specific gravity was recorded also.

In the case of milks made by the vacuum process, the raw milk was pre-heated in the hot-well until the temperature reached 200° to 210°F., after which it was drawn into the vacuum pan. The temperature in the pan ranged from 125° to 135°F., averaging about 130°F. The average vacuum obtained was about 23 inches. Unfortunately the available supply of raw milk was occasionally limited, with the result that the charges in the pan

were not constant in volume; as a consequence, the time of evaporation varied from forty-five minutes to one hour and fifteen minutes.

When the milk in the pan had reached a concentration of 7° to 9° Beaumé at 120°F., it was homogenized at 1800 to 2000 pounds pressure. Specific gravity, solids and fat were determined on the homogenized evaporated product after which one half of the evaporated milk was placed in sterilized bottles, of 250 cc. capacity, and these bottles were placed on ice until needed. Each bottle usually contained sufficient milk for one day's feeding.

The other half of the evaporated milk was also placed in similar bottles, plugged with cotton and sterilized at 240°F. for ten minutes and at 225°F. for five minutes, using a laboratory autoclave. These bottles were also placed on ice until needed for feeding purposes.

By the use of the analytical data, described above, it was possible to dilute the evaporated milk samples with distilled water until the diluted milks contained solids and fat equal to that of the raw milk from which they were made. All evaporated milks were fed daily in the diluted state in direct comparison with fresh raw herd milk.

EVAPORATED MILK MADE BY THE AERATION METHOD

During the early part of the investigational work the aerated evaporated milks were made in a Ruff evaporator of the commercial type, consisting of a heated revolving drum which was partly immersed in the milk. Air was driven through the heated milk by means of blower pipes. The experimental milk, used in this study, was produced by the college dairy herd that furnished the local market with certified and grade A milk. As a result there were periods when the amount of milk available for evaporation purposes was so limited that the quantity of milk was insufficient to immerse the heated drum. For this reason the use of the Ruff evaporator was abandoned and air pipes were connected with a glass lined pasteurization tank of small capacity and the subsequent aerated evaporated milks were made by this method, heating the milk by means of steam.

The hot-well temperatures were maintained at 145°F. and the condensing temperatures ranged from 130° to 155°F.   On account of the varying quantities of fresh milk, the time of condensing varied from forty-five minutes to one hour and fifteen minutes.

From this point the evaporated milks made by the aeration methods were treated the same as those made by the vacuum process; i.e., homogenized and a portion sterilized by the fractional sterilization method.

During the entire period the college herd was confined to the barn and yard, without pasture, and received a ration relatively constant in composition with the exception that some green hay was fed during two months in the fall.   The ingredients of the ration, expressed in parts per 100, were as follows:

	Per cent
Wheat bran	12
Barley	12
Oats	8
Hominy	24
Cotton seed meal	12
Linseed meal	16
Peanut meal	16

Some minor modifications were made in the proportions of the above ingredients during the latter part of 1924.   During the entire period mixed hay and silage were fed, as roughage, with exception of the two months mentioned above, when green hay was fed.

It will be seen, therefore, that the vitamin B intake of the herd was about as constant as can be expected under ordinary feeding conditions.

It will be noted that five types of experimental milks were fed, which we have abbreviated for convenience (on the figures) as (a) R, i.e., raw milk; (b) V.E., i.e., vacuum evaporated milk (not sterilized); (c) V.E.S., i.e., vacuum evaporated milk which had been sterilized; (d) A.E., i.e., aerated evaporated milk (not sterilized); and (e) A.E.S., i.e., aerated evaporated milk which had been sterilized.

THE FEEDING EXPERIMENTS

A total of 289 rats have been used in this work, although the charts do not represent that number, owing to the fact that it was necessary to repeat some of the early work on account of the observations of Steenbock, Sell and Nelson (6) and Dutcher and Francis (7) that vitamin B studies are inaccurate unless provisions are made to eliminate the possibility of the rats having access to their own excretory material. This error was eliminated in part at least, by changing our feeding technique and adopting the use of false cage bottoms consisting of wire screen (3 or 4 meshes to the inch). Since the publication of the above mentioned findings Salmon (8) and Smith, Cowgill and Croll (9) have published experimental data substantiating the work of Steenbock, Dutcher and co-workers and showing that this change in feeding technique is necessary if the best results are to be obtained. In our publication (7) we stated that we were of the opinion that "the rat is limited in its ability to store vitamin B." As a result it was decided that little could be gained by using the "curative" method of feeding and, consequently, all rats described in this paper were fed the various experimental milks from the beginning of the experimental period, rather than adding the milk to the diet after vitamin deficiency had become evident, which is the practice in the "curative" method.

RAT RATION AND FEEDING TECHNIQUE

The ration consisted, in parts per 100, of casein 18, salts 3, agar 2 and dextrin 77. Four drops of a potent cod liver oil were fed daily, separate from the ration, to insure an adequate intake of vitamins A and D. Iodine was furnished in the drinking water. The casein was prepared by precipitating skim milk according to the Zoller method (10). The washings, with acidified water (pH 4.8), never numbered less than 12 and between washings the casein was pressed as free of water as possible, in a cheese press.

The water washings were followed by 5 or 6 alcohol washings, allowing each "batch" to remain in contact with alcohol for at least three hours. This treatment served to extract any traces

of vitamin B remaining in the casein and, incidentally, removed sufficient water to prevent spoilage of the casein on standing. After treatment with alcohol the casein was spread in thin layers on glass shelves in an air drier and air (at room temperature) was driven over the casein to remove the alcohol.

The casein was then treated with ethyl ether until 50 cc. of the ether extract left no fatty residue, upon evaporation. The casein was then air dried, heated in an air oven at 120°C. for twelve hours and ground to a powder. This product has been used in all of our feeding work and has been found to be free from vitamins A, B and C.

The dextrin was prepared by autoclaving corn starch in the moist state, in the presence of 0.4 per cent citric acid. After drying in a steam closet, until the product was thoroughly brittle, it was ground to a fine powder.

The salt mixture was McCollum's salt mixture No. 185 and the agar was a high grade product of the type used in bacteriological work.

As is the custom in this laboratory, all experimental animals were confined in individual cages and food consumption records were obtained on every individual. These food intake records were used as a guide in the interpretation of data but are not of sufficient importance to be included in the charts.

Each experimental group contained not less than 8 individuals and some groups (particularly the groups receiving 12 cc. of milk) contained as many as 16 rats. Males and females were distributed equally in all groups to eliminate the influence of sex on the growth curves obtained in averaging the individual growth curves in each group. On account of the large number of animals, it was thought best to conserve space by presenting "group averages" rather than growth records of each individual. It was felt, also, that the data are more easily interpreted by consulting averages for each group.

All feeding experiments were conducted for one hundred forty days. In order that seasonal variations, if any, might be eliminated all animals were not placed on experiment at one time but groups were added from time to time during the experiment.

When this was done, however, an equal number of rats were placed in each experimental group.

Osborne and Mendel (11) have shown that a minimum of 16 cc. of milk, produced under conditions existing at New Haven, were needed to furnish sufficient vitamin B for the normal growth of rats. The work of Kennedy and Dutcher (2), working with milk produced under feeding conditions existing in St. Paul, found that they could obtain satisfactory growth of rats on as little as 10 cc.

With these results in mind, it was planned to feed the milk, described in this paper, at levels below and above 10 cc. Consequently, the milk was fed at 6, 8, 10 and 12 cc. levels with the hope that we would find a "threshold level" at which point vitamin destruction (if any) by heat or oxidation would be most marked.

DISCUSSION

All of the experimental data have been averaged and are summarized in figures 1 and 2. None of the animals receiving 6 cc. of milk grew normally, although growth was quite satisfactory.

No outstanding differences seem to exist between the groups receiving 6 cc. of raw milk and those receiving equivalent amounts of vacuum evaporated (V.E.) milk. Furthermore, the sterilization of this evaporated milk did not seem to affect its nutritive properties. The rats receiving 6 cc. of the aerated (A.E.) evaporated milk did not grow so well but the differences are not marked. Sterilization of this evaporated milk did not bring about differences sufficiently large to merit emphasis.

What has been said for the rats receiving 6 cc. of milk can be said, also, for those fed at the 8 cc. level. In fact, no effect of the additional 2 cc. of milk can be noted on any of the curves.

When the volume of milk is increased to 10 cc. it is possible to note a distinct improvement in all groups. It is difficult, however, to note any effect of milk treatment in any of the groups receiving more than 6 cc. of milk.

When the volume of milk, fed per rat, was increased to 12 cc. fairly normal growth was obtained, showing that the Pennsyl-

vania herd milk was slightly superior, in vitamin B content, to that described by Osborne and Mendel (11) but it seems slightly inferior to the Minnesota milk described by Kennedy and Dutcher (2).
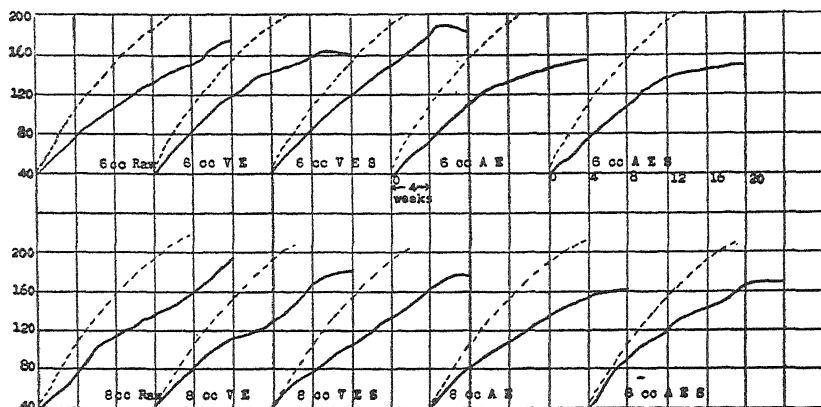
Fig. 1
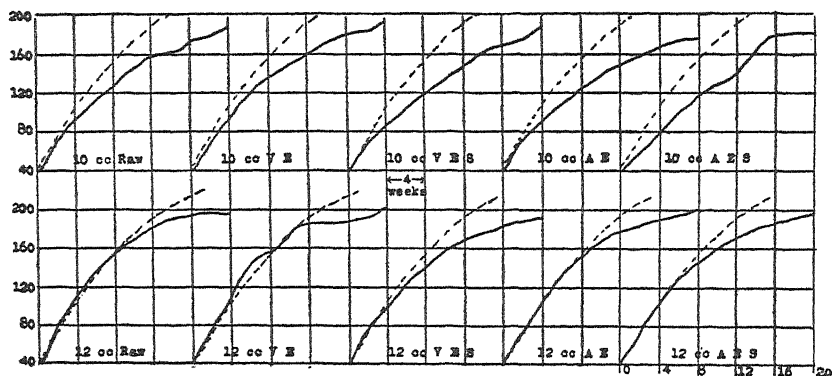
Fig. 2

SUMMARY AND CONCLUSIONS

Evaporated milks have been made by vacuum and aeration methods, approximating commercial manufacturing conditions as closely as possible. These milks were sterilized and compared, by animal experiments, month by month, with unsterilized evaporated milk as well as with the raw milk from which they

were made. This was done to study the effect of heat and oxidation on the stability of vitamin B. The evaporated milks were diluted with distilled water until they possessed the same composition as the raw milk and all experimental milks were fed at 6, 8, 10 and 12 cc. levels. No evidence of vitamin B destruction could be noted except in the groups fed at the 6 cc. level. In these groups there was some evidence that aeration or oxidation and heat may have brought about slight destruction of vitamin B. The destructive effect, however, was so slight that it is doubtful if it can be considered to be of nutritional importance.

We are forced to conclude, therefore, that vitamin B is not readily destroyed by the evaporation methods described and only under unusual conditions would we expect the vitamin B deficiency of commercial evaporated milks to be due to methods of manufacture.

Experimental work is now in progress to attempt to determine the stability of vitamin A during the processes of evaporation and sterilization by the methods described.

The writers wish to acknowledge the assistance of Miss Mattie Creighton, who assisted materially in the experimental phases of the work, and the hearty coöperation of the herdsman, Mr. P. D. Jones.

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THE EFFECT ON MILK PRODUCTION OF FEEDING MORE THAN THE HAECKER, ECKLES, AND SAVAGE REQUIREMENTS*

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Haecker (1) and Eckles (2), in their studies of the feed requirements for milk production, determined the amount of nutritive energy necessary to maintain milking cows at uniform body weight. Savage (3) has published a standard in terms of protein and total digestible nutrients, which is in substantial agreement with the results of Haecker and Eckles as well as with later results obtained at the United States Bureau of Dairying experiment farms, Beltsville, Maryland (4) (5). This last named standard will be used in describing the results to be reported in the following article.

The Savage standard gives the quantities of digestible nutrients necessary to maintain milking cows at uniform body weight. The question remains, however, whether cows will not produce more milk if fed more than this standard demands.

LITERATURE

Several stations have carried out work in which more feed was given in one period than in another. Two types of experiments furnish such data: one studying the effect of varying amounts of grain, and the other studying the effect of grain in addition to roughage.

The New Mexico Station (6) reports two trials of adding grain to a ration of alfalfa hay, and found an increase of 12.2 per cent in production. A study of light versus medium grain feeding

was made at the Utah Station (7), with a resulting increase of 8 per cent in production with the heavier grain feeding. In a study on "Heavy versus light grain feeding for dairy cows," the California Station (8) found an increase in production of 5 per cent on the more liberal ration. In all these trials the less favorable rations were supernormal, yielding digestible nutrients from 12 to 45 per cent in excess of the Savage standard requirements. Thus in each case there was an increase in production when an already liberal ration was made more liberal.

Other stations have made somewhat similar reports, but the experiments examined were so conducted as to have little bearing on the question of the effect of feeding at standard as against above standard. All these experiments were purely economy trials, and in but few cases apparently was an attempt made to regulate the quantity of nutrients to the need of the cows.

Eckles and Palmer in well regulated experiments studied the effect of the plane of nutrition on milk yield. In their first paper (9) they conclude that "only in certain cases, which are brought out in the data to be presented later, did an increase in the plane of nutrition above normal raise the flow of milk, and the influence in these cases was very limited." Their conclusion was that in the early months of lactation milk is controlled by a "chemical stimulus" or "hormone" present in the blood and that in this period the plane of nutrition has little if any effect. In the latter part of the lactation period, they conclude that the "chemical stimulus" largely disappears, that the "nervous stimulus" then controls milk secretion, and that here over-feeding does help slightly in holding milk production to a higher level.

These conclusions were based on five experiments in which cows were fed from 15 to 100 days at a supernormal plane of nutrition. A cow overfed for 65 days increased from 13.3 to 15.1 pounds per day. A cow overfed for 30 days increased from 22.5 to 22.9 pounds per day. A cow overfed for 100 days increased from 10 to 11.6 pounds per day. A cow overfed for 15 days increased from 9.4 to 12.9 pounds per day. A cow overfed for 30 days decreased from 20.3 to 18.7 pounds per day.

In a second paper by the same authors (10) on the effect of

underfeeding on milk production, by a similar train of reasoning
it is maintained that in the early months of lactation the plane
of nutrition—even underfeeding—affects but little, if any, the
level of milk production. In the latter stages of lactation the
experiments do show clearly a decided decrease in production
as a result of underfeeding, and the authors urge the impor-
tance of supplying sufficient feed after the "chemical stimulus"
imparted at the time of parturition has been lost.

These findings were based on 16 experiments in which cows
were underfed for varying periods, usually from 15 to 30 days.
These experiments fell into three distinct groups; those starting
at calving, those starting about 30 days from calving, and those
starting at a considerably later time after calving. In the first
group, in which all the cows were overfed before calving and then
underfed, three increased slightly in production while three
decreased. In the second group two cows, previously underfed,
were more severely underfed and decreased in production. The
other two cows, also previously underfed, were continued below
normal but not so severely underfed as previously and increased
in production. In other words they were given more feed,
though not a sufficient amount, and increased in production as a
result. In the third group six cows, all previously well fed,
decreased, some very considerably, in production during the
underfeeding period.

Further light on the effects of changes in feed on milk produc-
tion is to be gained from the reaction to feed changes of certain
cows in the study of Cary and Meigs (11) on the "Relation be-
tween the diet, the composition of the blood and the secretion
of milk in dairy cows." The experiments cited were mostly of
three periods of varying lengths. One cow starting about 30
days from calving, after a period on nearly an adequate ration,
had both the protein and energy reduced for 37 days and de-
clined in milk production from 12.25 to 7.80 kgm. per day.
When placed again on the full ration the decline stopped, and
the daily production was increased in 17 days from 8.26 to 9.03
kgm. A second cow 60 days from calving, after a period in
which she was fed nearly a sufficient amount, had only the

energy of her ration reduced for a period of 27 days and decreased in production from 11.11 to 9.77 kgm. A slight increase in production was noted in the third period when the displaced energy was returned to the ration. A third cow started 90 days after calving had merely the protein of the ration reduced in the middle period. Here too the production was reduced in the intermediate period and later increased when the ration was again made sufficient.

DISCUSSION OF THE ECKLES AND PALMER RESULTS

That element of the results of the investigation of Cary and Meigs above cited which deals with the effect on production of a ration insufficient to maintain body weight is intentionally compared with the work of Eckles and Palmer, as the results of Cary and Meigs are not entirely in harmony with the conclusions of Eckles and Palmer. The latter authors, as above stated, suggest that neither overfeeding nor underfeeding has an appreciable effect on milk production in the early stage of lactation when the "chemical stimulus" or "hormone" is active and that overfeeding even in the latter stages of lactation has but very small effect.

On the other hand the results of the investigation of Cary and Meigs show that cows even in the first months of lactation are quite susceptible to an inadequate energy supply. Their work also shows that an inadequate supply in either quantity or quality of protein is reflected in the milk flow even in the first 90 days of lactation.

Table 1, which is a portion of the table presented by Eckles and Palmer (10), forms a basis for the discussion of the lack of harmony between the two pieces of work.

From this table it will be noted that in the first group, which consists of cows starting the underfeeding period immediately after calving, the three most heavily underfed actually decline in milk production. The three most lightly underfed do increase somewhat during the underfeeding period. In the second group, consisting of cows starting the underfeeding period 20 to 40 days from calving, the two most lightly underfed before

the experiment and most heavily underfed during the experiment declined in production, while the other two increased in

TABLE 1

Shows the effect of a subnormal plane of nutrition on the milk yield during the first months of lactation

EXPERI-MENT	COW	DAILY MILK YIELD		STAGE OF LACTATION	UNDER FEEDING	PLANE OF NUTRITION	
		At first	At end			During experiment	Before experiment
		pounds	*pounds*	*days*	*days*	*per cent*	*per cent*
1	20	22.2	19.9	1	30	−60–70	Not given (?)
2	206	28.4	27.1	1	22	−60	Supernormal
3	301	23.6	16.0	3	30	−38	Supernormal
4	300	35.8	38.9	1	20	−35	Supernormal
5	2	12.3	13.7	4	38	−20–25	Supernormal
6	301	25.1	37.2	5	18	−16	Supernormal
7	2	13.5	8.8	42	36	−30–35	−20–25
8	301	35.3	33.0	24	20	−24.5	−16
9	20	19.6	26.1	32	16	−23	−60–70
10	206	26.1	27.1	23	12	−20	−60

TABLE 2

Shows the average daily milk yield of seven cows by ten-day periods from the time of calving

10-DAY PERIOD	cow 27	cow 62	cow 206	cow 304	cow 400	cow 303	cow 211
	(1)	(1)	(2)	(2)	(2)	(2)	(2)
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
First	29.3	11.6	40.3	36.7	20.8	29.5	39.6
Second	31.0	14.0	42.7	38.9	21.3	30.8	38.6
Third	32.5	14.7	43.3	39.8	22.8	30.3	37.1
Fourth	31.4	15.6	40.9	38.8	22.9	29.3	38.0
Fifth		15.0			22.7	29.7	40.4
Sixth					22.4		41.9
Seventh					22.6		42.4

(1) From Missouri Station, Research Bulletin No. 2.   (2) From Missouri Station, Research Bulletin No. 7.

production. However, there appears to be a reason for the improved production of these last two cows. The underfeeding of these cows was drastic before the experiment and much lighter

during the experiment. In other words, there was a raising of the plane of nutrition, and the cows responded to the improved conditions.

During the first few weeks of lactation under adequate feeding the milk yield generally increases considerably. This is shown in table 2 prepared from data of Eckles (12), and Eckles and Reed (13).

In every case the production was lower in the first ten days than in some of the subsequent periods.

Recognizing that this is the case, it would seem that any condition that prevented the normal rise in production would indicate the unfavorableness of that condition. In most of the cases reported by Eckles and Palmer there was not only an arresting of the rise in milk yield due to underfeeding but actually a decline that could be attributed to that cause.

As is well known the curve of normal milk production becomes a declining curve a few weeks after calving. If this fact is taken into account in a study of the data submitted by Eckles and Palmer (9) on the effect on milk production of a supernormal plane of nutrition, it would appear that excess feed makes a very appreciable difference in the milk yield. In four of the five experiments reported by them, there was not only an arresting of the normal decline for those cows in advanced lactation but an actual increase in production due to the overfeeding.

THE EXPERIMENTS

After the decision had been reached that the Savage feeding standard for maintenance and milk production furnished very approximately the amount of feed necessary to maintain uniform body weight, the next logical question was the effect on production of feeding in excess of this standard. Two types of experiments were conducted. The first were comparatively short time experiments, with periods of thirty days in which the cows were alternately fed at standard and above standard. The latter were whole lactation-period comparisons.

TABLE 3

Showing details of weight, production, feed received, and plane of nutrition according to the Savage feeding standard in the first experiment

| PERIOD | COW | BREED* | MILK | TEST | BODY WEIGHT | GAIN IN BODY WEIGHT | FEED RECEIVED | | | | PERCENTAGE OF SAVAGE REQUIREMENTS RECEIVED† | |
							Grain	Hominy	Alfalfa	Silage	D.P.	T.D.N.
			pounds	per cent	pounds	pounds	pounds	pounds	pounds	pounds	per cent	per cent
(1) At standard........	228	H.	322.1	3.40	1,410	-2	50.0	60.0	219.5	1,197.0	105.3	103.3
	236	H.	520.6	3.50	1,095	-29	115.8	38.0	209.5	1,111.0	103.1	101.9
	250	H.	664.4	3.70	1,201	18	158.0	54.0	236.0	1,170.0	100.6	100.4
	420	J.	263.7	5.05	689	2	88.0	17.0	147.0	726.0	106.5	96.5
	435	J.	271.4	5.10	689	5	60.0	74.0	138.5	762.0	102.5	105.3
	84	G.G.	433.8	4.30	816	3	108.2	62.0	170.0	846.0	101.0	100.3
Total........			2,476.0			-3	580.0	305.0	1,120.5	5,812.0		
Average........				4.00	983						102.5	101.0
(2) Above standard........	228	H.	246.6	3.80	1,393	-30	39.6	59.2	300.0	1,144.5	130.5	115.1
	236	H.	474.6	3.90	1,101	41	203.4	8.8	230.0	1,105.0	128.8	113.1
	250	H.	660.5	3.70	1,227	34	307.6	27.4	240.0	1,096.0	131.6	115.1
	420	J.	233.2	5.40	713	45	119.4	36.2	150.0	780.0	131.1	115.6
	435	J.	250.5	5.65	708	35	127.6	48.2	150.0	740.0	129.0	114.4
	84	G.G.	404.8	4.66	825	15	195.2	43.4	180.0	840.0	130.0	114.8
Total........			2,270.2			140	992.8	223.2	1,250.0	5,705.5		
Average........				4.30	995						130.2	114.6

(3) At standard	228	H.	192.0	3.90	1,373	−10	14.0	95.6	192.0	979.5	104.4	101.2
	236	H.	301.9	4.20	1,110	−23	40.8	90.4	205.0	948.3	104.8	104.3
	250	H.	611.0	3.95	1,243	−2	124.4	112.0	243.0	976.0	99.3	99.4
	420	J.	220.2	6.05	774	18	46.8	60.8	146.0	777.7	100.9	101.0
	435	J.	236.7	6.35	733	13	51.6	61.6	150.0	780.0	99.4	100.0
	84	G.G.	292.1	5.25	831	−2	48.3	64.4	184.0	832.0	100.2	100.0
Total			1,853.9			−6	325.9	484.8	1,120.0	5,293.5		
Average				4.75	1,006						101.3	101.0
(1) and (3) at standard, average			2,164.9	4.33	995	−5					102.0	101.0
(2) Above standard			2,270.2	4.30	995	140					130.2	114.6

Average increase in milk production of the above standard period over the standard period, 4.9 per cent.

* Breed: H., Holstein; J., Jersey; G.G., Grade Guernsey.

† D.P., digestible protein; T.D.N, total digestible nutrients.

First experiment

In this experiment six cows were fed for three periods of thirty days each. In the first and third periods they were fed as nearly as possible at Savage requirements, while in the second period they received 30 per cent more protein and 15 per cent more digestible nutrients than is required by the Savage standard. The protein was regulated by the addition of hominy feed to the basal grain ration consisting of hominy feed 2 parts, ground oats 2 parts, wheat bran 2 parts, linseed-oil meal 1 part, and cottonseed meal 1 part. Because the cows were far advanced in lactation no transition period was allowed between the experimental periods.

The feed allowed for maintenance was based on three-day body weights taken at the start of each experimental period. The feed allowed for production was based on the actual milk production and the fat test of a two-day composite taken at the middle of each ten-day subperiod. In other words, the feed allowed for production for each thirty-day period was based on the fat tests of three two-day composites taken at ten-day intervals. The results of the first experiment are shown in table 3.

In this trial cows that had been milking 18, 13, 11, 11, 9, and 6 months respectively and producing as little as would be expected at these stages of lactation increased on the average 4.9 per cent in milk production when fed 15 per cent in excess of Savage requirements for total digestible nutrients. It will also be noted that on the average the cows lost only 5 pounds in body weight per thirty days when fed at Savage requirements. This is a little less than one pound per cow.

Second experiment

In this experiment five cows were fed for three thirty-day periods with ten days between for transition. In the first and third periods it was planned for the cows to receive the Savage requirements, while in the second or intermediate period they were to get 20 per cent protein and 10 per cent total digestible nutrients in excess of Savage requirements. In other respects,

this experiment was conducted in exactly the same way as the first experiment. The results of the second experiment are shown in table 4.

In this trial the cows were about as far advanced in lactation as were those in the first trial, having been fresh 13, 13, 12, 11, and 7 months respectively. The cows were fed close to the planned amounts, both in the at-standard and the above-standard periods. The increase in milk production was but 1.9 per cent.

It will be noted that in these two experiments the percentage gain in production was quite small; 4.9 per cent in the first, and 1.9 per cent in the second. The average for the two experiments gives but 3.3 per cent actual increase in production. A more accurate index of the value of the supernormal ration is seen in the decline in milk yield under the two conditions. In chart 1 the average daily milk yield has been computed for all cows in both experiments by five-day subperiods, and the decline has been plotted for the two periods at standard feeding as well as for the intermediate period when fed above standard. This chart also gives the same information for the two experiments separately, each showing fairly close agreement with the combined chart.

It will be seen that at standard the cows declined nearly 20 per cent in the thirty-day periods, while at above standard there was no decline in production. In fact, in every five-day subperiod when the cows were fed above standard the yield was higher than in the last five-day subperiod when fed at standard.

From a study of the curves given in chart 1, it is clear that simply comparing the total amounts of the milk yield of the period when the cows were fed above standard with those of the preceding and subsequent periods when they were fed at standard does not give a fair quantitative idea of the effect of the surplus feed on the milk yield. On account of the large amount of feed given in the middle period, the cows start the third period at a higher level of milk yield than would have been the case if they had been fed at standard all along; in other words, a considerable part of the milk given in the third period

TABLE 4

Showing details of weight, production, feed received, and plane of nutrition according to the Savage feeding standard in the second experiment

| PERIOD | COW | BREED* | MILK | TEST | BODY WEIGHT | GAIN IN BODY WEIGHT | FEED RECEIVED | | | | PERCENTAGE OF SAVAGE REQUIREMENTS RECEIVED† | |
							Grain	Hominy	Alfalfa	Silage	D.P.	T.D.N.
			pounds	per cent	pounds	pounds	pounds	pounds	pounds	pounds	per cent	per cent
(1) At standard	50	G.H.	751.8	4.00	1,206	−33	152.0	65.6	300	1,200.0	100.0	100.2
	248	H.	1,003.2	3.70	1,192	−2	241.2	65.6	299	1,200.0	99.7	99.9
	416	J.	247.7	5.40	873	17	41.8	51.2	170	900.0	99.6	99.6
	427	J.	299.2	5.30	709	40	68.8	52.0	146	780.0	97.1	97.7
	443	J.	234.3	4.90	618	26	48.4	36.0	138	714.0	100.5	98.9
Total			2,536.2			48	552.2	270.4	1,053	4,794.0		
Average				4.25	920						99.5	99.4
(2) Above standard	50	G.H.	763.9	4.10	1,203	26	300.0	61.5	257	1,072.5	120.3	109.9
	248	H.	978.0	3.80	1,204	28	366.2	40.6	299	1,118.9	119.6	109.4
	416	J.	227.9	5.40	893	24	87.3	44.1	169	891.5	119.7	109.1
	427	J.	282.9	5.95	741	24	131.4	53.6	150	772.5	120.7	110.2
	443	J.	184.6	4.80	657	51	56.4	38.6	150	720.0	120.7	110.2
Total			2,437.3			153	941.3	238.4	1,025	4,575.4		
Average				4.37	940						120.2	109.8

(3) At standard	50	G.H.	782.9	4.30	1,204	−22	208.2	119.2	239	1,033.0	100.3	100.5
	248	H.	956.1	3.95	1,202	−33	207.0	118.4	298	1,038.0	96.8	98.1
	416	J.	194.4	6.03	901	−9	23.8	56.6	170	900.0	100.3	100.4
	427	J.	184.4	7.45	768	29	45.6	55.6	150	779.0	101.6	99.3
	443	J.	130.8	5.85	691	17	9.4	44.0	147	720.0	104.3	101.2
Total			2,248.6			−18	494.0	393.8	1,004	4,470.0		
Average				4.65	953						99.8	99.7
(1) and (3) at standard; average			2,392.4	4.44	937	15					99.6	99.6
(2) Above standard			2,437.3	4.37	940	153					120.2	109.8

Average increase in milk production of the above standard period over the standard period, 1.9 per cent.

* Breed: H., Holstein; J., Jersey; G.H., Grade Holstein.

† D.P., digestible protein; T.D.N., total digestible nutrients.

ought really to be credited to the surplus feed given in the second period.   A better idea of the true relations can be given
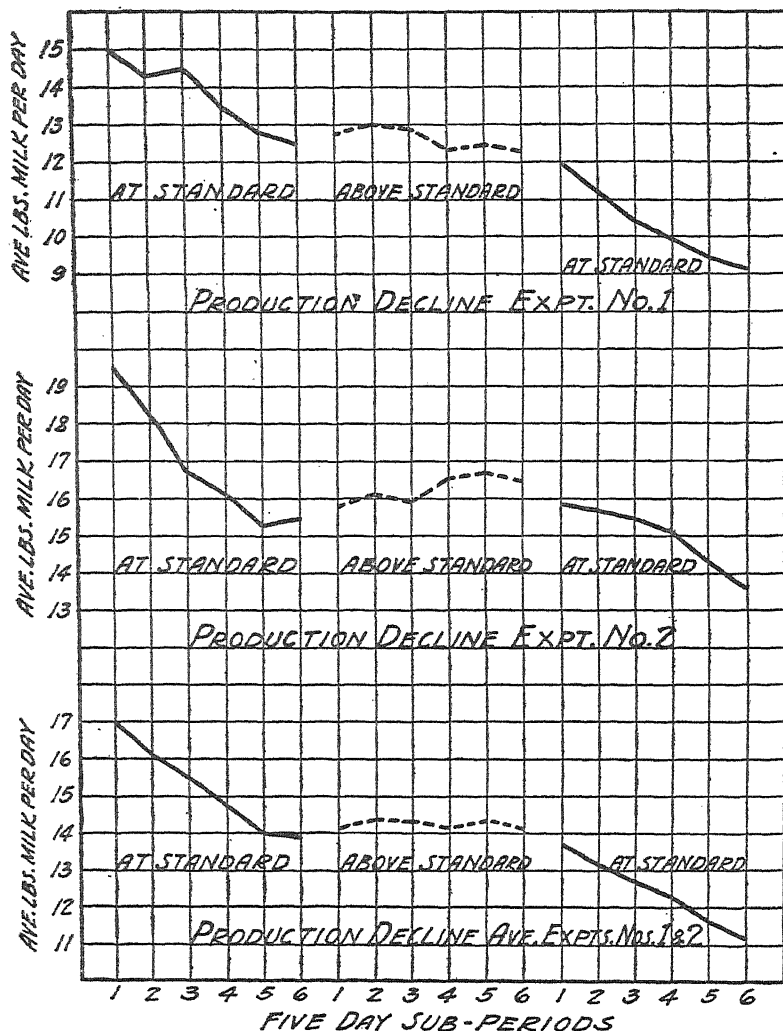
CHART 1

by comparing the actual milk yield of the second period with what would have been the yield if the decline had been as rapid

as it was in the first and third periods. On this basis of comparison, the surplus feed caused an increase of about 16 per cent over what would have been the yield if the cows had been fed at standard in the second period.

TABLE 5

Summary of third experiment, showing comparative results of two lactations on six cows when fed above and at standard

COW	AGE		LENGTH OF LACTATION	LENGTH OF PREGNANCY	PERCENTAGE RECEIVED OF SAVAGE REQUIREMENTS (T.D.N.[a])	MILK PRODUCTION	DECLINE IN MILK, FIRST TO LAST MONTH	PERCENTAGE DECLINE IN MILK PER MONTH	DAYS DRY BEFORE LACTATION
	years	*months*	*days*	*days*	*per cent*	*pounds*	*per cent*	*per cent*	
236	2	3	330	0	116.7	12,513.4	31.3	3.9	First lactation
236	4	8	303	201	101.3	11,654.3	65.2	8.2	80
255	2	9	365	215	113.3	11,202.2	34.9	3.5	First lactation
255	3	11	365	192	101.1	10,670.0	73.3	7.3	52
444	2	5	365	214	105.9	9,553.6	33.7	3.4	First lactation
444	3	7	365	218	99.8	9,157.1	64.2	6.4	62
442	3	3	331	154	122.4	6,567.3	77.2	8.6	114
442	4	7	331	209	100.3	8,088.4	82.0	9.1	85
443	3	0	336	148	126.8	6,680.9	68.4	7.6	86
443	4	3	336	209	100.6	7,828.2	52.1	5.8	46
465	6	10	365	176	121.9	8,411.7	60.4	6.0	Over 90
465	8	1	365	2	102.6	6,388.7	64.0	6.4	82
Average above standard........	3	5	344	151	117.8	9,154.9	49.0		
Average at standard.............	4	10	344	172	101.0	8,964.5	68.0		

T.D.N., total digestible nutrients.

Third experiment

Many cows at this station are placed on 365-day semi-official tests. They are kept in boxstalls, milked three times a day, and fed considerably above the requirements of the Savage
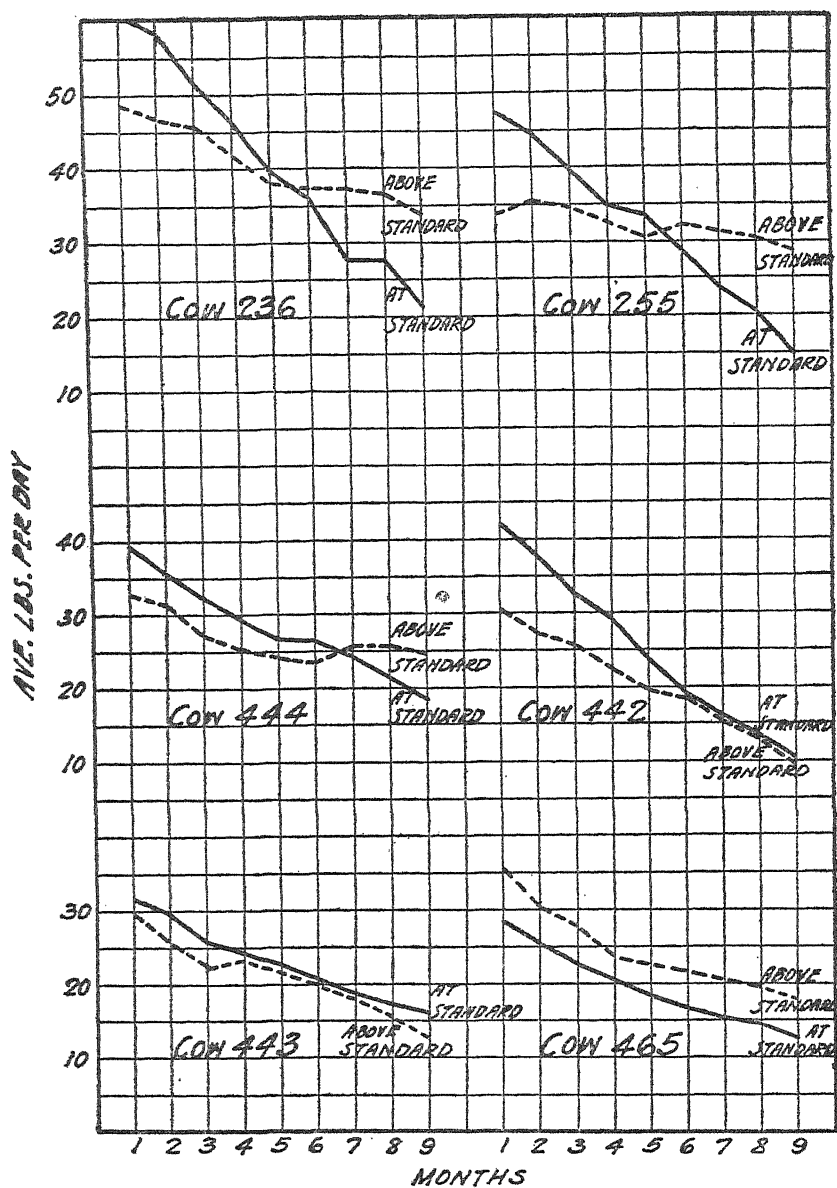
CHART 2

standard. Six of these cows were placed in the succeeding lac-
tation under the same conditions and their feed regulated as
nearly as possible to meet exactly the Savage standard.

In three cases, due to early conception, the second or at stand-
ard lactation had to be stopped at less than a year. In these
cases comparisons are made with an equal portion only of the
first or above standard period. Thus the two lactations for each
cow are of the same length. Table 5 gives the results of this
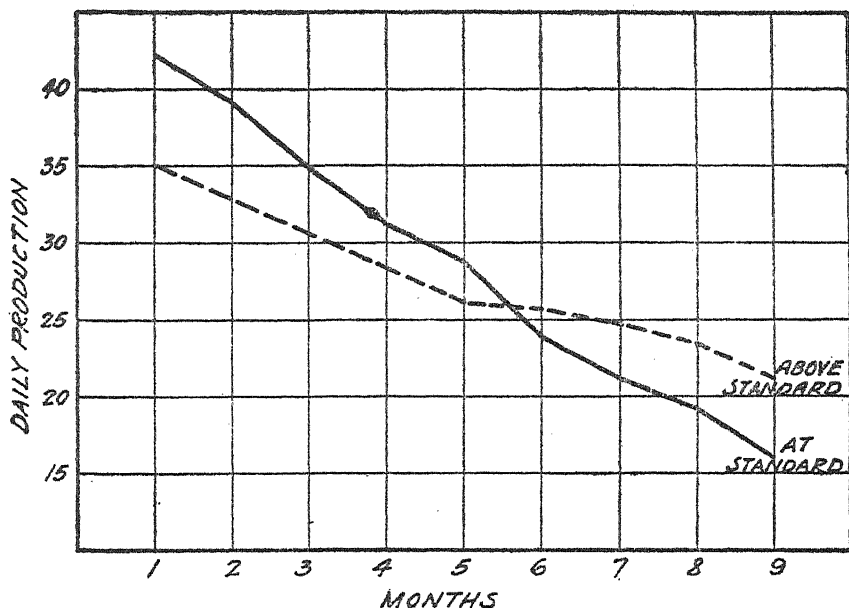
CHART 3

experiment, showing the comparative milk production, feed
received, and decline in production for each cow under the two
levels of feeding. The table also gives the time when the cows
became pregnant, their ages at the beginning of the lactation
periods and the length of time each cow was dry between lacta-
tion periods.

Chart 2 shows the decline in average daily milk yield for each
cow for the first nine full months of the lactations compared.
Chart 3 shows the average decline for the whole group.

From Table 5 it will be seen that the cows gave 2 per cent less milk when fed at standard than when fed above standard. They were pregnant for 21 days longer on the average in the lactation at standard, but were 1 year and 5 months older. They were all at a period of life when, other things being equal, they would have been expected to give more milk at the more advanced age.

Graves and Fohrman (14) give figures from which the increase in milk yield with advancing age in heifers can be calculated; and Ragsdale, Turner and Brody (15) give figures for the decrease to be expected as a result of pregnancy.

If the figures of Graves and Fohrman are applied to the milk yield of the cows in table 3 it will be found that, based on the production at 3 years and 5 months, the group should have averaged 10,288.6 pounds in the lactation at 4 years and 10 months in place of the average of 8964.5 pounds actually produced. This would show an increase of 15 per cent which they should have made if they had been fed above standard. It will be remembered that in the at-standard lactation the cows were pregnant on the average 21 days longer than in the preceding lactation. From the figures given by the Missouri investigators above referred to it would seem that an allowance of 4 per cent for this fraction of a month would be a very liberal one for the extra time pregnant. This would be less than 1 per cent for the lactation. Thus stated quantitatively it would appear that feeding at standard produced approximately 14 per cent less milk than would have been produced if the cows had been as highly overfed in the second lactation as in the first.

The comparative decline in production in the two lactation periods as shown in charts 2 and 3 is another way of indicating that feeding cows just at standard does not produce the maximum milk yield. Only one of the six cows decreased more rapidly when fed above standard than when fed at standard, and that difference was but slight as compared with the difference in the opposite direction of several of the other cows. Four of the cows decreased decidedly more rapidly when fed at standard, as was the case in the short time experiments. A comparison

of the graphs of cow 236 and cow 255 is of especial interest.   Cow 236 was not pregnant during her first lactation, yet was pregnant 201 days in the second or at standard lactation.   The more rapid decline in production in that lactation might be attributed to pregnancy but for the fact that cow 255 showed declines very similar to those of cow 236, with length of pregnancy just slightly greater in the at-standard lactation.   If the cows in the third experiment had declined in milk production no more rapidly in the second lactation than they did in the first, they would have produced nearly 16 per cent more milk than they did when fed only at standard.   Thus figured in two ways, the value of the overfeeding in this experiment was about 14 per cent and 16 per cent respectively.

SUMMARY

The results taken together indicate that feeding cows according to the Savage or Haecker standard, that is, so that they will just maintain uniform body weight, does not keep them at their maximum milk yield.   The three experiments cited are in close agreement in showing that an above standard ration very materially increases production.   The two short time experiments with cows well along in lactation give a 16 per cent increase in milk yield as the result of feeding 12 per cent more than the requirements; while the third experiment in which whole lactation period comparisons are used, showed from 14 per cent to 16 per cent increase in production as the result of feeding 17 per cent more than the requirements.

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DAIRY NOTES

THE FUMIGATION OF CHEESE BY HYDROCYANIC ACID GAS

In the JOURNAL OF DAIRY SCIENCE (viii: 287–289, May, 1925), there appeared an article by Perez Simmons entitled "Hydrocyanic acid retained by fumigated cheese." This paper gives the authors' experimental data on the fumigation of cheese, which are the basis for his criticism of the recommendation for fumigation practice as given in California Agricultural Experiment Station Bulletin 343 (May, 1922) by E. R. deOng and C. L. Roadhouse. Mr. Simmons states that "until more extensive tests are made to prove otherwise, in regard to each variety of cheese, cyanide fumigation should not be recommended." But he fails to recognize the fact that the bulletin referred to dealt with only one type of cheese, cheddar, and that in his own data which he cites, this variety absorbed but "a mere trace" of hydrocyanic acid gas.

The experiments performed by deOng and Roadhouse were on this one type of cheese only, as reported in the bulletin: tests on other varieties were purposely omitted as they are of little commercial importance in California.

Toxicity tests made by deOng and Roadhouse on white mice by allowing them to eat freely of the freshly fumigated cheese showed no ill effects whatever. In later tests the experimentors themselves ate from one to three ounces of the fumigated cheese, both that enclosed by the rind and freshly cut surfaces within one hour after removal from the fumigating room but without any ill effect.

Later experiments by the Federal Insecticide and Fungicide Laboratory as reported in United States Department Bulletin 1307, pages 6 and 7, show that American cheese when first removed from the fumigatorium might have as high as 110 parts per million of hydrocyanic acid gas but this drops to 55 p.p.m. in one day and 22 p.p.m. in seven days. The authors state that "the hard rind of an American Swiss cheese was enough, however, to prevent the gas from penetrating. None was found even close under the rind, although some was present in the rind." The authors failed to discuss their findings from the standpoint of toxicity and made no tests from this standpoint.

Hence, the authors of California Bulletin 343 conclude both from the data contributed by Perez Simmons and E. L. Griffin et al., as well as their own findings that their recommendations for the use of hydrocyanic acid gas as a fumigant for cheddar cheese should stand as given.

E. R. DEONG AND C. L. ROADHOUSE.

THE VITAMIN B REQUIREMENT OF THE CALF[1]

S. I. BECHDEL, C. H. ECKLES AND L. S. PALMER

Five vitamins are now known to be important in human and animal nutrition. Some of them are more important in the life of certain species than they are in others. For example, the antiscorbutic factor does not appear to be necessary in the ration of the calf, the pig, the rat, or the chicken, while it is essential in the ration of man or the guinea pig. On the other hand, the rat and the chick have rather heavy demands for the antirachitic factor while swine appear to tolerate a relatively low supply. The extent to which the results with laboratory animals can be applied to the larger domestic animals, is, therefore, questionable.

Eckles, Palmer et al. (1) studied the effect of yeast as a supplemental feed to rations ordinarily fed to calves from 20 to 180 days of age. The results were negative since no increase on the rate of growth or beneficial effect on the health of the calves could be observed. Eckles and Williams (2) made a similar study with lactating cows and obtained negative results. The outcome of these studies make it apparent that there is no advantage in feeding a vitamin B supplement to the rations commonly fed to calves and lactating cows in good dairy herds.

In a search of the literature no reference could be found concerning a systematic study to determine whether the growing calf requires a supply of vitamin B in its ration similar to that found necessary for the growth of laboratory animals. This

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paper is intended to set forth the results of an investigation which was designed to throw light upon this question.

Theiler and associates (3) have made a contribution in their studies of the disease in South African cattle known as lamzietke. In the course of their investigations rations consisting of polished rice supplemented with a small amount of autoclaved roughage (Veldt hay and oat straw) were fed to cattle for periods ranging from 44 to 52 weeks with no evidence of beri beri symptoms. Theiler suggested the possibility of cattle being able to synthesize vitamins by means of bacterial symbiosis in the digestive tract.

FUNCTION OF VITAMIN B

Vitamin B is commonly referred to as a growth promoting vitamin, although it possibly may include two factors, viz., the antineuritic vitamin, the absence of which causes polyneuritis in fowls and beri beri in man, and a water soluble growth promoting substance. Sherman and Smith (4) after studying all the investigations on vitamin B up to 1922, state that the absence of this factor from the diet causes cessation of growth, that a partial but not complete deficiency of this vitamin leads to impaired growth and a general undermining of health and vigor, and this lowered vitality may have a far reaching effect in its influence on reproduction and the successful rearing of young.

EXPERIMENTAL

The selection of a palatable ration that carries all of the known dietary factors for growth and well being of calves, excepting vitamin B, offers a real problem since all of the common hays and cereal grains as well as milk and milk powders are known to contain a considerable amount of this vitamin. The liberal use of by-product feeds offers the only solution.

It was expected that the employment of such a ration would involve other dietary factors, since none of the principles of vitamin nutrition had been applied to cattle or the other larger

domestic species at the time of starting the investigation in 1922. As was more or less expected some mistakes were made in the first attempts, and the early results are reported as preliminary experiments. The major part of the data is presented as the main experiments. The main portion of the study covers the period in the life of the calf between five and thirty months of age while a limited amount of data are presented on the period between birth and five months of age.

Preliminary experiments

Ration. Feeding trials on rats (5) had indicated that corn gluten feed and commercial dried sugar beet pulp, when added to the ration in such proportion as would be suitable for calves, carried little or none of the water soluble B growth factor. With this information at hand it was planned to formulate an experimental calf ration as follows:

Feed

	pounds
Corn gluten feed	100
Casein	50
Polished rice	50
Butterfat	25

A commercial grade of casein was used, as a more highly purified product would have been prohibitive. Polished rice is generally conceded to be devoid of vitamin B, while butterfat is considered a good source of vitamin A. At this time (1922) definite information on the anti-rachitic factor was not available, so that no provision was made for supplying it in the ration.

The butterfat in a melted condition, after being decanted, was mixed with the concentrate mixture. A quantity sufficient to last for about two weeks' feeding was prepared at one time. Dried beet pulp was depended upon solely to supply roughage.

Two bull calves, one a Holstein, S-3, 8 months of age, and the other a Jersey, S-6, seven months of age, were selected for the preliminary trial. These animals, about normal in size, had been raised on the usual skim-milk and grain ration. They were confined in box stalls and fed the experimental ration in a dry

condition in such amounts as they would consume from day to day. Records of the feed consumption, live weights at ten day intervals, and height at withers at 30-day intervals were kept on these as well as subsequent experimental animals, but for the sake of brevity these data are omitted.

The two animals got along nicely on the ration for about 100 days. They then began to exhibit signs of stiffness and did not have full control of their legs in walking. They also went off feed. The addition of vitamin B (10 per cent of dried yeast) to the ration of the Holstein, S-3, failed to give any relief, and at 113 days on experiment he went entirely off feed. He had a severe form of scours and at this time it was discovered that he was almost blind. A white spot appeared on the center of one eye ball. A profuse mucous discharge ran from his nose almost continuously. Fourteen days of the yeast treatment failed to afford any relief, and wheat germ, $\frac{1}{2}$ pound daily produced no improvement. The daily feeding of the juice of two oranges to supply vitamin C was tried without results. On the 120th day fit-like spasms occurred, in which he would fall down and be unable to get back on his feet for a time. The administration of two ounces daily of cod liver oil brought almost immediate improvement. He came back on full feed, and when taken off the experiment at 220 days he was 122.4 per cent normal in weight. The cod liver oil was continued for a period of 18 days but the wheat germ was continued to the end of the experiment.

Results with the Jersey bull, S-6, were much the same as those exhibited by the Holstein. He continued along for about two months gradually showing more and more signs of malnutrition. He displayed the same profuse nasal discharge as the Holstein. On the 166th day of the experiment he was given cod liver oil in two ounce daily doses. At about this time it was discovered that he had pneumonia. The cod liver oil was discontinued after ten days feeding and daily doses of five pounds of wheat germ were given. Five days later the juice of two oranges were administered daily. He gradually recovered and came back on full feed, but was again stricken with pneumonia and taken off the experiment.

At the time it was impossible to draw any conclusions from the results obtained on these two preliminary animals. No definite proof was obtained that the addition of vitamin B to the ration offered any relief. The addition of cod liver oil appeared to afford the most relief, but all of the credit could not be assigned to it since vitamins B and C were administered at about the same time. Further comment, and what is now believed to be a satisfactory explanation, will be presented following the discussion of results on the second preliminary experiment.

Second preliminary experiment

A repetition of the preliminary trial with a larger number of animals appeared to be the next logical step. The ration employed was the same, except for the addition of the following minerals:

    12.0   pounds calcium carbonate (precipitated chalk)
    1.5   pounds acid potassium phosphate
    0.875   pound ferric citrate
    0.313   pound potassium iodide

Animals. Four grade Holstein animals, approximately 8 months of age were selected. Three of them, B-4, B-10, and E-5 were bulls. One, E-6, was a free martin. They had been fed the usual skim-milk, grain, and hay ration.

Calves E-5 and E-6 received the experimental ration without supplements. Tomato juice and wheat germ to supply vitamins B and C were supplements to the ration of B-4 which was selected as a check animal. Two ounces of cod liver oil were added daily to the ration of B-10 in order to get further information on its apparent curative effect on the calves in the previous experiment.

Results of second preliminary experiment. Animal B-10 that received the cod liver oil supplement continued along on the ration for over a year and made most excellent growth and development. He was found normal in every respect when slaughtered at the close of the experiment. He showed no signs of vitamin B deficiency at any time in his career.

Animal B-4 thrived well until on experiment 108 days when he developed a stiffness in all parts of his body. He almost entirely refused to eat; his knees and ankles became swollen; and he developed a tendency to stand with his hind legs forward, so that his body was in a squatty position. He appeared to give much evidence of rickets as observed in other animals. Daily doses of 2 ounces of cod liver oil were given. About two months previous to this time, on account of repairs being made at the experimental barn, the animals were confined in the main barn. In these quarters they did not get daily out-of-door exercise as they did before. In the light of our present understanding of the importance of sunlight in nutrition, it would be reasonable to suppose that this condition might never have arisen had out-of-doors exercise been provided continuously.

The cod liver oil was increased to 4 ounces daily after five days and this amount was continued throughout the experiment. Inprovement was observed in a few days, but it came about very slowly. The animal did not eat well, especially of the grain. On account of the unpalatability of the wheat germ it was left out of the ration on the 146th day of the experiment. The daily doses of 350 cc. of tomato juice being fed at this time were believed to be sufficient for any needs of vitamins B and C that this animal might have. He ate the ration more freely after the wheat germ was left out and on the 158th day was much better. He did not get back on to full feed until the 220th day of the experiment. All signs of the rachitic condition had disappeared by this time. At 320 days on experiment he was up to normal in weight and above normal in height. When put on an alfalfa hay and grain ration he gained more rapidly than on the experimental ration, but his gains were not phenomenal. When slaughtered no indication of abnormal condition could be found. In view of the results before and after feeding cod liver oil, the lack of antirachitic factor is the most plausible explanation of the conduct of this animal.

E-5 made fairly good gains for the first 220 days on experiment. His appetite declined then, and his growth rate slowed up. Throughout the experiment he at times exhibited stiffness

in walking. He developed a profuse nasal discharge early in the experiment, and this persisted through his whole career. At times this condition became exceedingly bad. On the 316th day he began to show signs of a general swelling in all four legs. It so happened at this time that the butterfat had been unavoidably left out of the ration for about one week. The addition of the butterfat made the ration more palatable and he began eating. Sixty-gram daily doses of marmite were administered and almost immediate improvement resulted. He became apparently normal in a few days, but when the butterfat was again intentionally left out of the ration for one week, with the marmite continued, he again refused to eat, became very emaciated, and lost 17 pounds in weight.

From time to time it had been observed that the feed would tend to take on a lardy or tallowy-like smell. This indicated oxidation of the butterfat which adhered to the feed particles as a thin film. It was then surmised that possibly the ration had been at times deficient in vitamin A and perhaps antirachitic factor. This is the most plausible explanation of the deportment of this animal.

On account of E-5 not being very thrifty when put on experiment it was decided at the end of a year to put him on a good grain and hay ration to determine whether he would be able to thrive under such treatment. He was able to do this, and on slaughtering him 30 days later, no abnormalities could be detected.

E-6 made consistent growth for about 1 year. The profuse nasal discharge gradually grew worse toward the close of her career. At 370 days on experiment she refused to eat and developed a severe form of scours. The butterfat had been left out of her ration also for about one week when she became very weak and emaciated. She was given 10-ounce daily doses of cod liver oil with the result that the scours disappeared and she made considerable improvement for about two weeks. Even with the cod liver oil continued she again declined. Sixty-gram daily doses of wheat germ extract brought no improvement. She gradually grew weaker, refused to eat, and finally died on the 416th day.

On post mortem, a large number of abscesses were found in the kidneys and right liver. This condition alone was enough to cause death. Abscesses were also found in the lungs which adhered to the ribs and diaphragm. There were a few other minor abnormalities in addition to a chronic catarrhal condition in the sinuses of the head which were filled with mucous and pus. The bones and joints showed no abnormal condition.

Vitamin A deficiency best explains the results with this animal also. Although the cod liver oil brought only temporary relief, it is reasonable to assume that the deficiency could have been the indirect cause of the abscesses, and that the condition had developed so far when the cod liver oil was administered that a cure could not be effected. Furthermore, Jones (6) found that cod liver oil effected cures very slowly when calves were suffering from vitamin A deficiency.

The evidence when summarized tends to indicate conclusively that vitamin A was insufficiently supplied to five of the six animals used in the preliminary experiments. The outstanding symptoms of calves suffering from vitamin A deficiency as recently reported by Jones (6) parallel in several cases those exhibited by the calves in these preliminary experiments. The following are outstanding examples: The blindness and severe scours of the Holstein animal S-3; the profuse nasal discharge exhibited by all the animals except B-10; severe scours in the case of E-6; loss of complete control of the legs exhibited by several of the animals; and oedema in the case of E-5 and E-6.

The results with one animal, B-10, that had received cod liver oil continuously contributed evidence that calves can maintain normal growth and well-being without a supply of vitamin B in the ration. The data on the five other animals is considered valueless except for the fact that it paved the way for a more closely controlled main experiment.

MAIN EXPERIMENTS, PART I

In planning a continuance of the investigation provision was made for conducting a series of parallel experiments on rats with the view of obtaining first hand information on the vitamin

B deficiency of the calf rations employed. It was the aim also to use at least twelve calves and to get them started on the experimental ration at the earliest possible age. The use of heifer calves only was planned for the advantages that might be afforded in making a later study on reproduction and lactation.

Ration. In order to insure an adequate supply of vitamin A, plans were made for feeding cod liver oil as a supplement to the
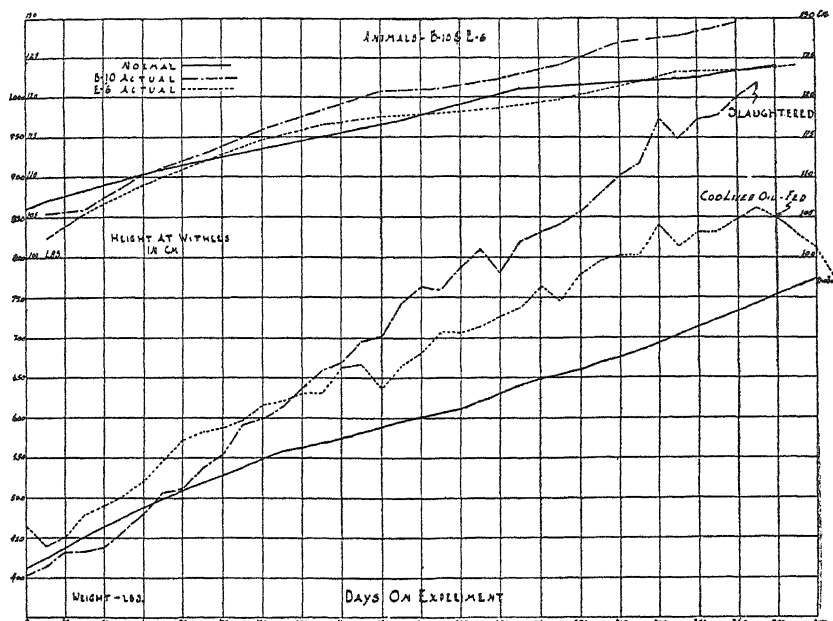
FIG. 1. GROWTH CHART ON TWO OF THE SIX ANIMALS USED IN THE PRELIMINARY EXPERIMENTS

Note the excellent growth made by B-10 on the vitamin B deficient ration supplemented with cod liver oil.

experimental ration. A high grade of medicinal oil was selected. It was kept in brown glass containers in cold storage, and provision was made for feeding it in fresh daily doses of 15 to 45 cc. per day, depending on the size and age of the calf.

Since the rations employed in the preliminary experiments were rather unpalatable to some of the calves, several changes were made to improve this defect. Imported dried beet pulp

(Holland) was found to provide a big improvement. It was cleaner than domestic pulp and was highly relished by the calves. The addition of cane sugar to the concentrate mixture made it possible to get further palatability by soaking the beet pulp and grain together for 12 hours before feeding. A high grade of casein put out by the Grove City, Pennsylvania, Creamery also provided further improvement in palatability. In the later months of the experiment, an imported product (Argentine) of equal quality took the place of it.

The ration was formulated as follows:

> 200   pounds corn gluten meal
> 100   pounds cane sugar
> 100   pounds commercial casein
> 62.5   pounds polished rice
> 62.5   pounds corn starch
> 24.0   pounds precipitated chalk
>   3.5   pounds mono-basic potassium phosphate
>   1.75 pounds ferric citrate
>   0.625 pounds potassium iodide.

Considerable amounts of pearled hominy from white corn were used to take the place of the rice and starch in the last months of the experiment. The ration was widened somewhat as the animals advanced in age, due account being taken for provision of protein and energy sufficient for growth.

The check animals were fed 40 grams per day of marmite to supply vitamin B. No provision was made for supplying vitamin C in the ration since the work of Thurston, Eckles and Palmer (7) had demonstrated this to be unnecessary. The cod liver oil carrying antirachitic factor in connection with the liberal mineral supply was assumed adequate for mineral requirements.

The amounts of feed consumed varied according to the size, age, and appetite of the calf. The minimum for young calves was about 1.5 pounds of concentrate and 2.5 pounds of beet pulp daily. The maximum amounts consumed daily by any animal when about 30 months of age was 5 pounds of concentrates and 10.5 pounds beet pulp.

Laboratory animal feeding tests. A series of eleven rat feeding

trials involving the use of 127 animals, fed individually in cages with screen bottoms (3 meshes to 1 inch), were conducted with the view of determining the vitamin B deficiency of the calf ration. In nine of the trials the calf ration was fed to young rats with certain modifications. It was not possible to feed beet pulp to an extent greater than 25 per cent of the total ration. On a 50 per cent beet pulp ration the rats would bloat and die in about one week. The scheme of feeding alco-
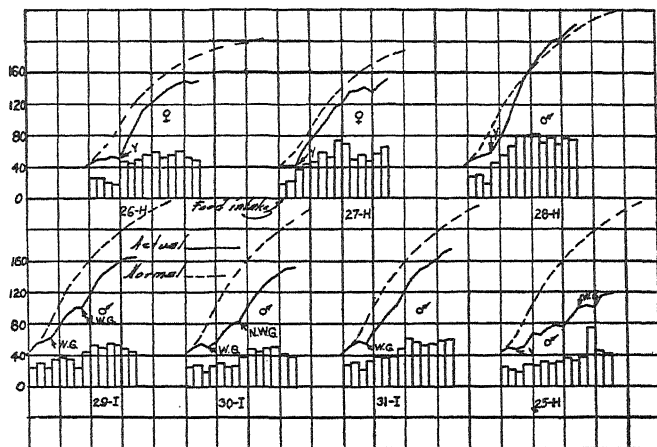
FIG. 2. FEED CONSUMPTION AND GROWTH BY WEIGHT OF RATS FED A RATION CONSISTING OF ALCOHOLIC EXTRACT OF BEET PULP, CALF GRAIN MIXTURE 75 PER CENT, AND AGAR 50 PER CENT

W. G. and N. W. G. indicate the addition of wheat germ extract to the ration, and Y. indicates the addition of yeast.

holic extract of beet pulp in amounts that would be equivalent to 50 per cent of beet pulp in the ration was resorted to in two trials.

In order to determine that beet pulp did not carry substances toxic to rats, two trials were conducted with pulp that had been subjected to extraction with alcohol. Two tests were made on the concentrate mixture alone. Agar and dextrin were supplemented to the extent of 5 per cent, and 20 per cent of the ration respectively in order to adjust the protein content and afford some bulk.

Very consistent results were obtained in all of these feeding trials. The calf ration, on account of vitamin B deficiency, would not support growth of the young rats for more than 2 to 4 weeks. This deficiency was proved through the feeding of wheat germ extract or yeast supplement which enabled the rats to recover, to thrive, and in many cases to attain normal size. A considerable number of controls gave conclusive evidence that the calf ration was nutritionally complete for the growth and well being of rats when it was supplemented with either yeast

FIG. 3. FEED CONSUMPTION AND GROWTH BY WEIGHT OF RATS FED A RATION CONSISTING OF CALF GRAIN MIXTURE, 75 PER CENT; DEXTRIN, 20 PER CENT; AND AGAR, 5 PER CENT

W. E. indicates the feeding of wheat germ extract

or wheat germ extract. A graphic summary of two representative feeding trials is submitted in figures 2 and 3.

Pearled hominy was tested for its vitamin B potency through feeding it, one case as 25 per cent, and in another case as 50 per cent of a synthetic basal ration. The fact that the hominy was practically devoid of vitamin B is indicated in figure 4 which summarizes one of these feeding trials.

Experimental calves. Four grade Holstein female calves, numbers 1167, 1168, 1169, and 1170, and four purebred Holstein female calves, numbers 1176, 1175, 1182, and 1185 were

used. The grades were designated as group I and the purebreds as group II. The ages at the time of going on experiment varied between 112 and 179 days, the calves in group I being the younger. Calves 1167, 1168, and 1169 had been fed a small amount of milk and were considerably under normal size at the time of going on experiment. All others had received care and treatment corresponding to the practice of good dairy farms, and with the exception of 1185 were about normal in size when put on experiment. Number 1185 was more or less unthrifty
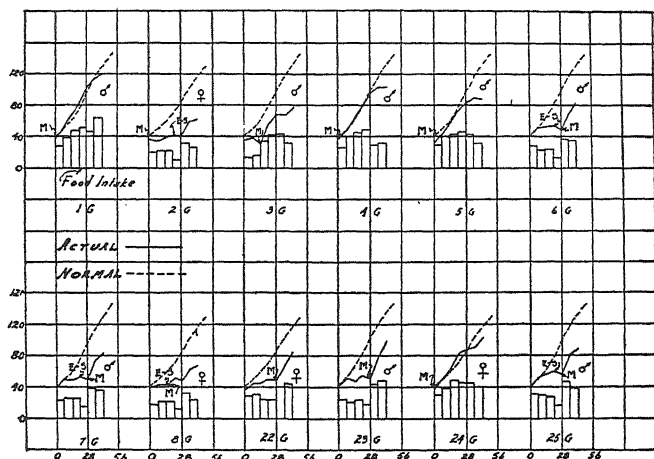
Fig. 4. Growth Curves on Rats Fed a Basal Ration Containing 50 Per Cent of Pearled Hominy

E. S. indicates the addition of wood shavings extract to the ration; M. the addition of marmite.

from birth. Numbers 1167, 1182, and 1185 were chosen as controls and were accordingly fed 30 grams daily doses of marmite in the ration.

Effect of vitamin B deficiency on the growth of calves

Figures 5, 6, 7, and 8 summarize in graphic form a record of the growth of the eight individuals in groups I and II. All of these heifers were continued on the experimental ration for periods ranging from 20 to over 140 days longer than the time indicated

on the charts. The growth rate portrayed on the charts was continued in every case.

The conduct of all the subjects was surprisingly uniform. They tended to eat sluggishly and thrive poorly for the first couple months. As a result, they were considerably under normal weight for the greater part of the first year on experiment. Even though Numbers 1167, 1168, 1169, and 1185, were handicapped in being below normal weight at the time of going on experiment, they, as well as the four other individ-
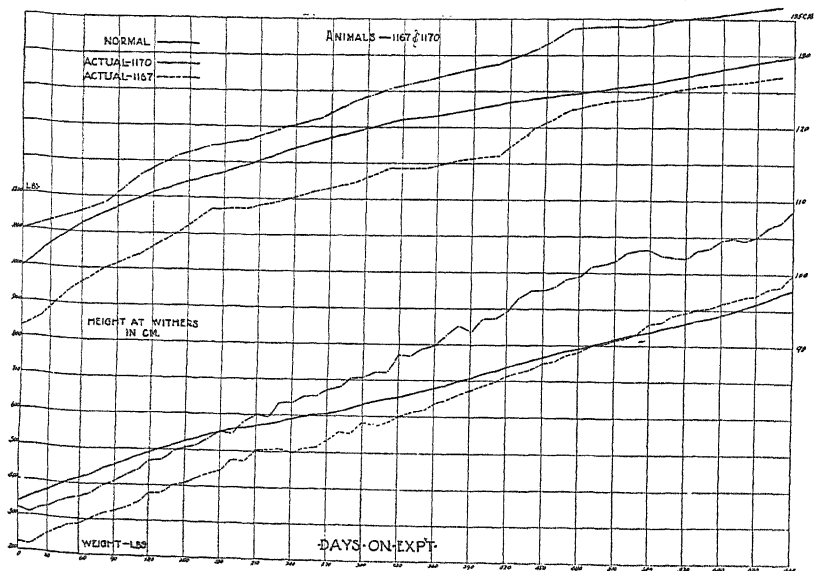
FIG. 5. GROWTH CURVES FOR 660 DAYS ON TWO CALVES FED THE RATION DEFICIENT IN VITAMIN B

uals, were able to overcome this handicap and to attain weights and heights at withers equal to or considerably greater than normal before they were two years of age.

At no time did any of the individuals exhibit any signs of vitamin B deficiency. The control animals gave no evidence of doing any better than the others, and the removal of marmite from their rations had no effect whatever on their growth or general well being.

The dried sugar beet pulp as the sole source of roughage appeared to be highly satisfactory for growth and general well being. The lack of bulk in the ration appeared to promote the habit of the calves to eat considerable wood shavings that was used for bedding and also to chew at the wooden partitions of their pens, especially in the earlier months of the experiment.
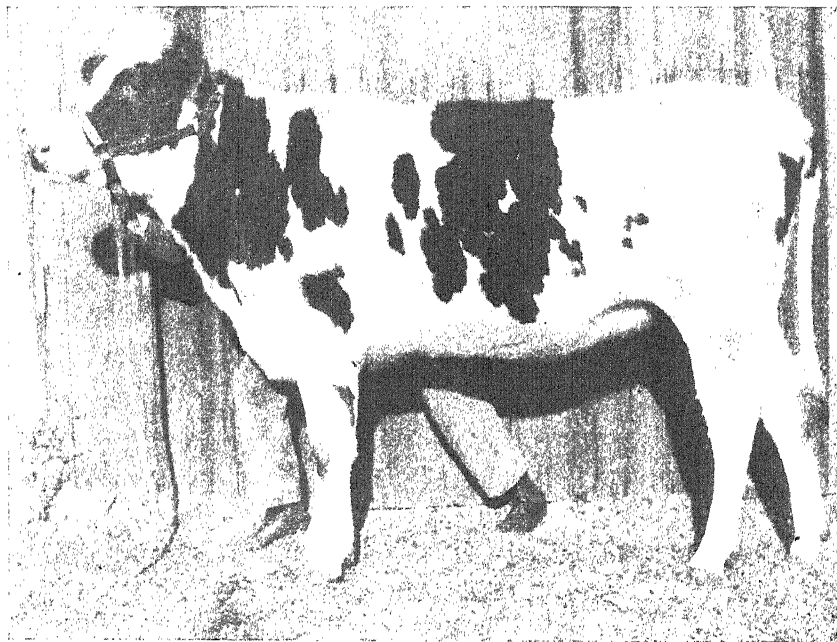
FIG. 6. ANIMAL 1170 AT THE AGE OF 27½ MONTHS WHEN ON EXPERIMENT 660 DAYS

Her weight, 1127.8 pounds, is 120 per cent normal and her height at withers is 105.7 per cent normal. She is a typical representative of the heifers in the main experiment when on the deficient ration 600 days or longer.

The fact that none of the animals ever ruminated was also another sign that the ration lacked bulk. The development of barrel in all cases appeared equal to that of heifers fed normal rations.

Four of the heifers when taken off the experiment and given a grain and hay ration began ruminating in about 24 hours after the change. It required about two weeks for them to come up

to a full feed of grain, hay, and silage. So far as can be determined, the feeding capacity was not injured by the experimental ration.

All of the heifers were bred and at the time of this writing (April, 1926) five of them have dropped strong, vigorous calves that are normal in every respect. Not a single animal failed to conceive. As they approached the time of parturition the mammary development became most excellent (see fig. 10).
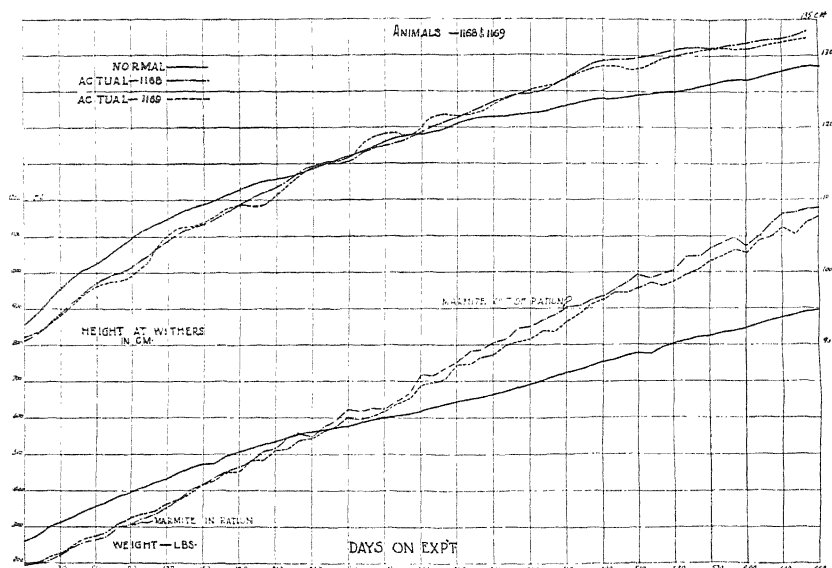
FIG. 7. GROWTH CHART FOR 660 DAYS ON TWO CALVES FED THE RATION DEFICIENT IN VITAMIN B

The marmite supplement in the ration of calf 1168 until the 445th day had no effect.

They were all very fat at this time, and their coats were quite sleek and glossy. The first three heifers to freshen, numbers 1168, 1169, and 1170 had considerable difficulty in calving and required assistance. This was perhaps due mostly to the fact that the calves were quite large, the birth weights being 107, 102, and 83 pounds respectively for the three heifers. They also had some difficulty with retained placentas, but finally cleared up satisfactorily in about one week.

They came back on full feed in a couple of days after freshening and then apparently went along in fine shape, the milk production being from 20 to 35 pounds per day. About the end of the second week of lactation they began going off feed. In about one week they refused to eat entirely. Sixty-gram daily doses of marmite given as a drench failed to bring any response. Drenches of 1½ pints cod liver oil and ½ pound of
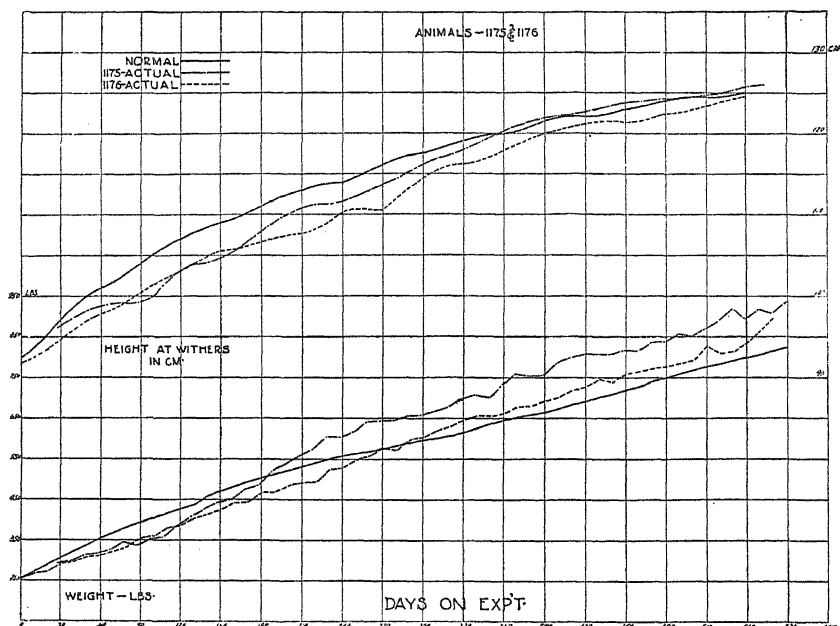
Fig. 8. Growth Chart on Two Calves Receiving the Ration Deficient in Vitamin B

precipitated chalk was later tried with the same result. In the case of heifers 1170 and 1182 prevention of the difficulty was tried through feeding three times the usual amount of cod liver oil beginning about two weeks before freshening. Number 1182 was also fed 60-gram daily doses of marmite, but these heifers almost exactly duplicated the action of 1168 and 1169. The only difference observed was that 1182 delivered her calf unassisted and dropped her placenta promptly.

A subnormal temperature of about $1\frac{1}{2}$ degrees was observed in all of them after they got entirely off feed. They became quite thin and weak, tended to get rather stiff, especially in their hind parts, and the milk production declined rapidly. The bowel movement ceased almost entirely. They consumed in some cases more than the usual amount of water and the urine flow was quite copious. There was a good deal to indicate that
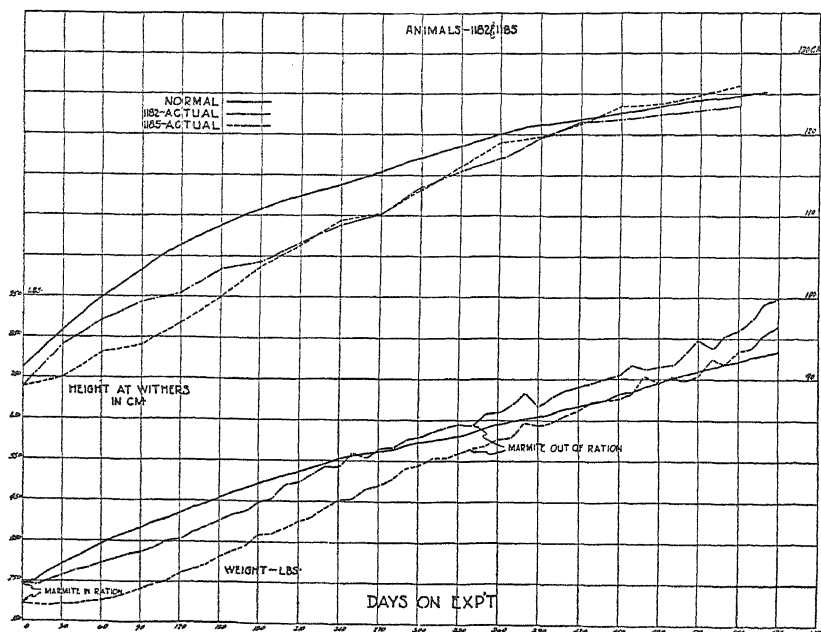
FIG. 9. TWO CALVES FED THE DEFICIENT RATION WITH MARMITE SUPPLEMENT FOR THE FIRST 340 DAYS

No stimulation of growth by the marmite could be observed, and when it was dropped from the ration the rate of growth was maintained.

the metabolic processes were quite low. It was with great difficulty that they were induced to eat small amounts of alfalfa, bran, oats, and oil meal, when these feeds were first offered to them. It was necessary to do some forced feeding with molasses dissolved in water. Once sufficient hay was eaten to start rumination, the appetite appeared to pick up rather rapidly.

That the difficulty is due to a mere lack of bulk or roughage in the ration, rather than to a mineral or vitamin deficiency, we are not in position to say. It appears that the trouble may be the same as that reported by Reed and Huffman (8), McCandlish (9) and other investigators (10).

The problem will be pursued on the other heifers due to freshen in the near future, and also on mature cows that are accustomed to dairy rations ordinarily fed.
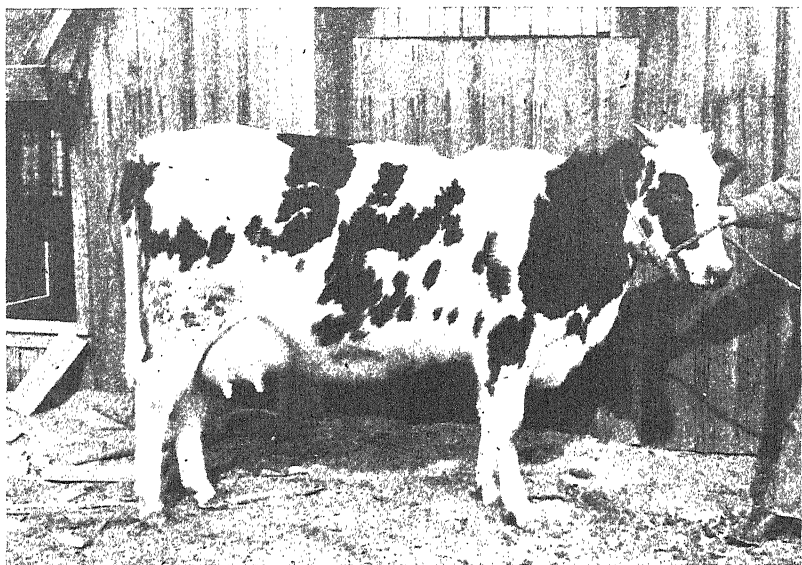
FIG. 10. ANIMAL 1182 THREE DAYS BEFORE SHE FRESHENED

She had been on experiment 690 days at this time, weighed 1180 pounds and her condition is representative of all the experimental heifers previous to freshening.

It had been the plan to raise the calves on their mother's milk until they could be weaned to the dry ration deficient in vitamin B. It was expected that the milk would be deficient in vitamin B and that the second generation could perhaps be grown up on a deficient ration. Unfortunately such was not possible. One calf was maintained on its mother's milk to the age of three weeks. It became quite vigorous and increased in weight

38 pounds in this time. In order to get definite information on the vitamin B potency of the milk, a large quantity was saved up and stored below freezing temperature before the heifers went off feed. Results of rat feeding trials with this milk, now in progress, will be published in a later paper.

MAIN EXPERIMENTS, PART II

The object of this experiment was to feed calves on a ration deficient in vitamin B from birth. The plan consisted in feeding cows due to freshen in several weeks on a ration deficient in vitamin B with the idea of continuing them on the same ration for a considerable period after freshening. Kennedy and Dutcher (11), McCollom, Simmonds, and Pitz (12), and Hughes, Fitch and Cave (13), have reported that vitamin B in milk is dependent on vitamin B in the diet.

In view of these data, it was assumed that the cows would produce milk, low in vitamin B, which could be fed to the calves until such time when they could be weaned over to the ration deficient in vitamin B which was described in part I.

Animals used. Two Jersey cows, 936 and 838, due to freshen in 6 weeks, and two Holsteins, 850 and 988, due to freshen in 10 weeks were used.

Care and feeding. Dried sugar beet pulp, the sole source of roughage, was fed with the following grain mixture:

> 200 pounds corn gluten meal
> 160 pounds pearled hominy from white corn
> 35 pounds tankage (60 per cent)
> 16 pounds special steam bone meal
> 2 pounds salt

All four cows ate the ration very satisfactorily at the start, and the Jerseys never got off feed until at or near freshening time. They were both brought back on feed by eliminating the tankage from the ration, but in about ten days after freshening 838 refused to eat entirely and finally had to be taken off the experiment. Cow 936 was in much better shape. She produced enough milk for both calves, 1222 and 1224, and did not

go off feed until about one week before the Holstein cows freshened. Every conceivable change of ration failed to bring her back on feed, and she died a few days later. The actions of these cows in refusing to eat after freshening were almost exactly like those of the heifers as described in part I. The one Jersey calf, 1224, was accidentally killed by the herd bull. The other, a heifer, 1222, was fed milk from the Holstein cows.

The Holstein cows resented the ration very much from the start. It was finally necessary to eliminate the tankage and
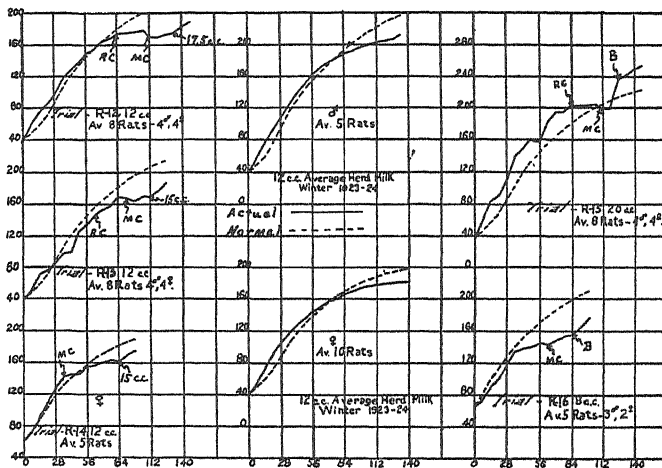
FIG. 11. GROWTH CURVES REPRESENTING AVERAGES OF GROUPS OF RATS IN MILK FEEDING TRIALS

R. D. indicates time of change of ration of cows 988 and 850; M. C., that milk was changed to that from cows receiving a good ration; 17.5 and 15 cc. that milk was increased to such amount; and B. that wheat germ extract was added to the ration.

bone meal. They lost considerable weight and were quite thin when they freshened. The calves, both heifers, were strong and vigorous, however. They were designated as 1230 and 1231.

About two weeks after freshening these cows went off feed just as the Jerseys had done. A change of ration was necessary to keep up the milk flow for the three calves. They were brought back on feed by supplementing the ration with oats and poor

quality, overripe, timothy hay which was three years old. The grain mixture from this time on consisted of two parts oats, one and one-half parts gluten meal, and one and one-half parts pearled hominy.

Rat feeding experiment. Five rat feeding trials were conducted for the purpose of determining the vitamin potency of the milk produced by the cows. The rats were confined in individual cages provided with screen floors (three meshes to one inch), and fed a basal ration consisting of 18 per cent purified casein, 3 per cent salts (McCollum 185), 2 per cent agar, and 77 per cent dextrin. Milk as the sole source of vitamin B was supplemented at different levels. Figure 11 gives a graphic summary of all five feeding trials.

In the first trial (R-12, fig. 11), the milk from the Jersey cows was fed for the first three weeks. Milk from cows 988 and 850 was fed during the remainder of the time except when a change to milk from cows receiving a good ration was made as indicated on the chart. In the next trial R-13, as well as in all subsequent trials only the milk from 988 and 850 was fed except as indicated. Feeding trial R-14 was started after the cows were advanced one month in lactation and when no further changes in the ration of the cows were necessary.

Twelve cubic centimeters of average herd milk had been about adequate for normal growth of rats in trials conducted in this laboratory during the previous winter. Two average curves of these data are included for convenience of study. These rats did not drop below the normal growth line until on experiment nearly 84 days, whereas those in experiments R-11, R-12, and R-13 dropped under the normal growth line somewhat earlier. Furthermore, at the end of 112 days they were considerably smaller than those fed normal herd milk for the same period of time.

The growth curve of the group R-15, fed 20 cc. made gains greater than normal and thus gave some indication that the milk was very little deficient in vitamin B. The group R-16, that received 8 cubic centimeters gave every indication of inadequate vitamin B supply, especially when wheat germ extract was supplied.

Although the data do not warrant strong conclusions, they indicate that it is possible to reduce the vitamin B content of cow's milk to some extent through feeding a poor ration. Although in agreement with data of Kennedy and Dutcher (11) the results are not nearly so outstanding as these investigators were able to obtain. Hart (13) has recently expressed an
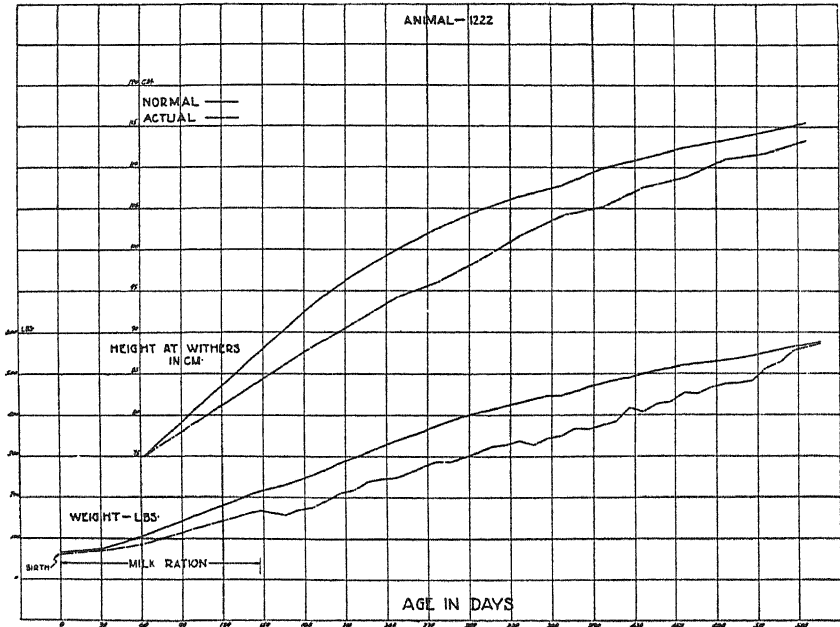
FIG. 12. EFFECT OF RATION DEFICIENT IN VITAMIN B ON GROWTH OF JERSEY CALF

Milk from cows fed a deficient ration was the main constituent of the calf ration for the first 147 days.

opinion out of harmony with our data. In commenting on grass versus winter feeding, he says that "Vitamin B is not very abundant in milk and is not influenced by the two types of feeding."

Effect of feeding calves a ration deficient in vitamin B

The three calves, 1222, 1230, and 1231, designated as group III, were fed on whole milk from the Holstein cows as their

sole ration until about 8 weeks of age.   At this time they
began eating the experimental concentrate mixture deficient in
vitamin B and some dried beet pulp.   The milk was discon-
tinued as indicated on the growth charts (figs. 12 and 13) and
the experimental ration was then fed after the methods out-
lined in part I.
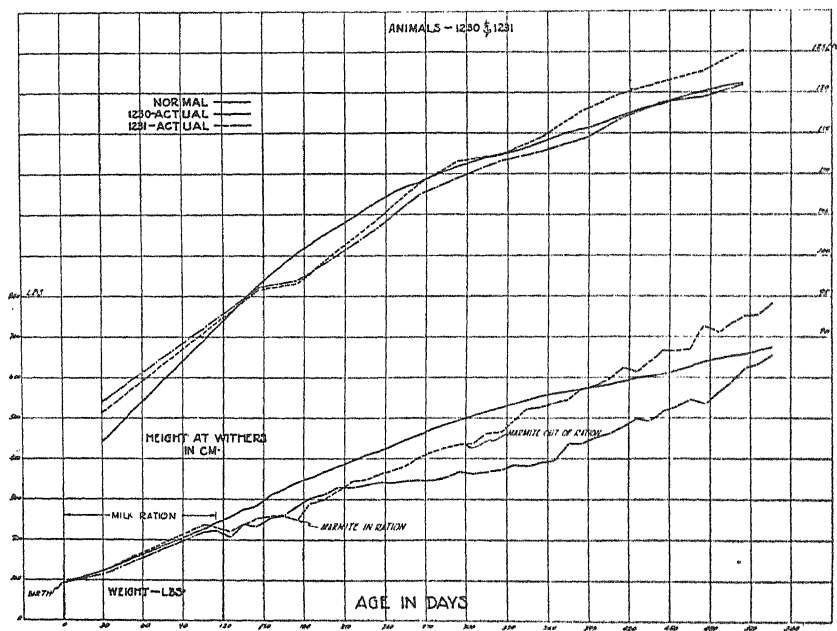
FIG. 13. EFFECT OF RATION DEFICIENT IN VITAMIN B ON GROWTH OF
HOLSTEIN CALVES

Milk from cows fed a deficient ration was the main constituent of the calf
ration for the first 115 days.

It is admitted that the practice of keeping calves on a ration
which was made up almost solely of whole milk for so long a
time was questionable, but no better plan seemed possible.   It
was our desire to bring them through if possible without the
usual hay and cereal grain supplements until such time when
they could get along on the experimental ration that was being
fed to the older calves reported in part I.   It was our feeling

that if this plan could be carried out, these three subjects would be superior to the older ones for a further vitamin B study.

They could not be induced to eat the experimental ration in sufficient quantities to warrant taking the milk away from them, and it was exceedingly difficult to get them weaned and going on the experimental concentrate and beet pulp ration alone, even when the Jersey was almost five months, and the Holstein nearly four months of age. They thrived throughout their milk ration career and exhibited no signs of deficiency disease. The Holsteins were very little under normal size at four months of age, but the Jersey at five months of age was less than 80 per cent normal in weight and 95 per cent normal in height at withers.

Group III was able to overcome the serious handicap of its early career and to attain about normal growth before the individuals were two years of age. The deportment of the group corroborated that of groups I and II in every way. They have been kept on the experimental ration for over four months beyond the time reported on the growth charts, and at this writing (April, 1926), 1230 is 109.4 per cent normal in weight and 103.3 per cent normal in height. Number 1222 is 98.7 per cent normal in weight and 98.5 per cent normal in height. Number 1230 will freshen in June, 1926, and the other members of the group are due about four months later.

GENERAL DISCUSSION

The results on growth and reproduction observed with the eleven animals reported in the main experiment are considered conclusively negative, but this should not be interpreted to mean that calves do not have a vitamin B requirement for growth and well being. It does mean, however, that it is either a case of no requirement for the factor in the ration, or such a small one that it cannot be measured with laboratory animals.

In view of the recent evidence put forth by several investigators we venture the opinion that bacteriological synthesis of vitamin B in the digestive tract suggested by Theiler (2) accounts for the fact that our experimental animals were able

to thrive, grow to maturity, and to produce normal offspring on a ration practically devoid of vitamin B. Damon (15) has made an outstanding contribution in this field of bacteriological research. He has been able to prove that several acid fast organisms synthesized vitamin B when grown in vitamin B free media. The dried bacterial cells fed to the extent of 7.5 per cent of a basal ration deficient in vitamin B stimulated rapid and continuous growth in young rats that were in a state of nutritive decline. Kuroya and Hosoya (16) have reported positive results with a pure culture of B. coli, but Damon's work does not confirm this. Heller, McElroy, and Garlock, (17) have produced evidence of bacteriological synthesis of vitamin B in the intestinal tract of the rat. Scheunert and Schieblich (18) have produced evidence of vitamin B synthesis by the organism B vulgatis commonly found in the intestinal tract of herbivora.

It is our plan, through the use of a fistula into the digestive tract of one or more of the remaining experimental heifers to obtain further light on the possible synthesis of vitamin B. This added phase of investigation in connection with the work now under way on the milk produced by the experimental heifers should give some light on the question.

Since dried sugar beet pulp was used as the sole source of roughage for all of the animals reported in this investigation, it is in order to comment on the so-called roughage requirement commonly mentioned in feeding practice. At this writing (April, 1926), nine of the calves, three of which have been on experiment since birth, have been maintained on the beet pulp ration for over two years. The barrel development has in every instance been apparently equal to that of calves fed on normal rations (see figs. 7 and 10). Evidence that the digestive tract was normally developed was obtained in a post-mortem on one animal, no. 1170. The rumen as well as other parts of the tract contained a large amount of food in the usual stages of digestion. The habit of eating considerable quantities of wood shavings, which were used as bedding, has continued with all of of the animals to the end of their careers. It is evident from this that the deficiency of bulk prompted the habit, even though,

reproduction, and general well-being were satisfactory. The absence of rumination in all of the animals throughout their careers was also likely due to the deficiency in bulk. These observations made it appear possible that the ruminating function could be effected by grinding hay and other roughages as advocated by certain manufacturers of feed grinders.

The inability of the heifers to lactate on the ration raises a question concerning the causation being due to a deficiency of roughage. Unfortunately, all experiments so far reported have dealt with calves. Reed and Huffman (8) have recently reported progress on rather extensive investigations. They have been unable to raise calves to maturity on milk alone, on milk and grain, or on grain alone. Calves fed solely on concentrates would die with convulsions and the post-mortem findings indicated toxemia. They attribute the toxemia condition probably due to a deficiency of a dietary factor rather than to crude fibre or bulk. Wheat straw, oat hulls, and wood shavings, did not prevent evidence of toxemia. Attempts to bring about normal development on milk rations without roughage were made through the feeding of calcium and phosphorus compounds. The results of these treatments prompted them to state that it is apparent that the irritability in calves fed on a concentrate ration is not due to a calcium or phosphorus deficiency.

Since our calves thrived so well through the growth period to maturity, it is evident that the beet pulp must have in some way supplied the deficiency encountered by Reed and Huffman. Our deficiency for lactation on the ration is probably the same or closely related to that observed by Reed and Huffman. If it is the same, the demands for lactation are evidently much heavier than for growth and reproduction. Since the present tendency in our high producing dairy herds is to supply nutrients more and more from concentrates rather than from roughage, the problem is of immense practical as well as of scientific importance.

Practical application of results

The average calf ration used in feeding practice is made up largely from leguminous hay and cereal grains. Since these feeds are usually classed as fair to good carriers of vitamin B, there is little likelihood of an ordinary calf ration being low in this vitamin. The present tendency toward raising calves with a minimum amount of milk would have scarcely no application since milk is, relatively speaking, not a heavy carrier of vitamin B.

Even though the fast growing tendency toward the feeding of by-products, especially of the milling industry, may eventually bring about the extensive use of rations which carry small amounts of vitamin B, the results of this experiment warrant the statement that the live stock man need have little concern about vitamin B deficiency ever standing in the way of his raising strong, healthy calves.

Furthermore, no evidence has been found to cause any fear, that the very serious problem of reproduction in our dairy herds will be further enhanced by vitamin B deficient rations.

CONCLUSIONS

1. An experimental calf ration composed of corn gluten meal, commercial casein, cane sugar, rice, pearled hominy, corn starch, dried sugar beet pulp, and a mineral mixture, carries an insufficient amount of vitamin B to support growth in rats and enable them to live for periods longer than 2 to 5 weeks.

2. A calf will grow normally to maturity and produce normal offspring on a ration that carries an insufficient amount of vitamin B to support growth and general well being in rats. Assuming that the calf possesses a physiological requirement for vitamin B similar to that of other animals, the deportment of the experimental calves described in this paper can be explained only on the basis of vitamin B synthesis by bacteria and other microörganisms in the digestive tract, unless future investigations should prove that various species of animals differ materially in their vitamin B requirements.

3. The milk produced by cows fed on a ration deficient in

vitamin B is appreciably, but not markedly, reduced in its vitamin B content.

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A COMPARISON OF GUERNSEY SIRES

II. BASED ON THE AVERAGE MATURE EQUIVALENT FAT PRODUCTION OF DAUGHTERS DURING THE MONTH OF MAXIMUM PRODUCTION*

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The large probable number of genes or factors included in the inheritance of the characters concerned in the secretion of milk and fat by the dairy cow has resulted in little progress being made toward the genetic analysis of these important characters. Some of the more simple characters of cattle such as coat color, skin color, horns, and a few abnormalities have been studied genetically and have been found to behave in a Mendelian fashion. A few workers such as Wilson (1) and von Patow (2) have advanced theories as to the number of factors concerned in the inheritance of milk secretion. Wilson proposes that there are 4 dominant factors which individually produce 20, 15, 10, and 5 pounds of milk daily and collectively 50 pounds and 4 recessive factors which produce 4, 3, 2, and 1 pound of milk and collectively 10 pounds of milk. This being true there would be 16 grades of milk yield varying from 50 pounds daily down to 10 pounds.

While the theory is suggestive, it is believed that a larger number of factors are concerned and without more exact data than is yet available, the factorial analysis of the milk secreting function of the dairy cow is impossible.

A promising mode of attack of this problem appears to lie in a further study of the mechanism or mode of milk and fat secretion during the lactation period and to separate into groups those series of factors which may influence or regulate certain characteristics of the lactation curve of milk and fat secretion. In other words, instead of attempting to study single factors, a

study would be made of groups of factors which together bring about certain results.

Sanders (3) has studied English Milk Records from the standpoint of the shape of the lactation curve. He has determined what is called the *shape figure* of the cow's lactation by determining the ratio of the total lactation yield to the maximum yield. This ratio is the same as the ratio which we consider to indicate the persistency of secretion during the lactation. This relation will be pointed out later in the discussion.

Considerable attention has been given to a study of the characteristics of the shape of the lactation curve at this Station. Studies have been made of some of the factors affecting the shape of the rising segment of the curve (4), the production at the maximum (4), and the declining segment of the curve (5). These investigations have indicated that of all the factors concerned in the lactation yield of fat two are outstanding in their influence.

The first of these is the height of production of milk and fat during the maximum month or other convenient unit of time. The second is the persistency of secretion or the rate of decline of production. These may later be further subdivided as knowledge of the mechanism of milk secretion increases.

Data has already been presented giving information on the mode of inheritance of yearly fat production in the Guernsey breed (6). It is the object of this paper to present similar data on the inheritance and mode of transmission of fat production during the month of maximum production using the same yearly records.

FAT PRODUCTION DURING MAXIMUM MONTH OF LACTATION PERIOD

Before considering the results of the study, it might be well to briefly consider the factors concerned in governing or limiting the maximum secretion of the dairy cow. The milk and fat which a cow is capable of secreting under favorable conditions of feeding and management in a given length of time is largely determined by two considerations. These are (1) the size of

the udder and the amount of secreting tissue present, and (2) the rate of secretion by a unit of secreting tissue. Do cows having the same amount of secreting tissue vary in their daily production of milk due to differences in the rate of secretion? Does the relation between the storage space and secreting tissue of the udder change the rate of secretion? Does the amount of blood passing through the gland influence the activity and rate of secretion? Is the size of the cow correlated with the size of the mammary gland or does the size of the animal influence production by increasing the capacity of the cow for feed consumption and greater blood supply or both?

The answers to these questions would greatly aid in clarifying the problem. While we do not have direct evidence by which the above questions may be answered a number of experiments have been conducted which furnish an indirect basis for making certain deductions. It has been shown by work at this Station that there is considerable variation in the activity of the mammary gland during a period of twenty-four hours as measured by the variation in the yield of milk during two-hour periods (7). This is believed to be due to the variation in the volume and nutrient content of the blood passing through the udder. It seems probable that mammary gland cells have an upper limit in their secretory activity which may vary among cows due to their inheritance with the result that certain cows although having equal secretory tissue would be able to produce more milk in a given interval.

It has been clearly shown also that the frequency of emptying the udder bears a close relation to its secretory activity (8). Does not this indicate that the amount of storage space in relation to secretory tissue would regulate or govern to some extent the rate of secretion. In other words, the inheritance of the size and internal structure of the udder would have a direct influence on the yield during the time of maximum milk flow.

Data is also available on the relation of the size of the cow to total yearly production (9). It was found that with animals of the same age an increase of 100 pounds in live weight produced an average increase of 20 pounds of fat per year. As

blood volume is correlated with live weight (about 5 pounds per 100 pounds), it is certain that more blood is available for milk production. That more blood actually passes through the gland, however, is not known. It would appear likely, however, in view of the greater production unless body size is positively correlated with udder size. This data indicates the extent of the influence of size inheritance on total yearly fat secretion. A greater proportionate relation to maximum fat secretion would undoubtedly be shown by live weight. Size inheritance must, therefore, be considered in its relation to maximum fat secretion. It is believed that the number of genes concerned in size inheritance must be considered as a part of the total inheritance for fat production and not as certain workers evidently believed that the inheritance of milk and fat secretion is confined to the stimulus for secretion (10).

Maximum secretion is the resultant of many anatomical and physiological relationships. Summed up they consist of gradations of size of the mammary gland both of secretory tissue and storage space, size of the digestive organs which furnish nutrients for the blood, size of the body in general which may be indicated by blood volume, and a stimulus to rapid production of the secretion. The variation in this stimulus probably will best be shown by the correlation between the summation of these size factors and maximum production. A high degree of correlation between the physical conformation of the dairy cow usually associated with large production and the yield of milk and fat will indicate that the rate of secretion in unit time does not vary greatly; a low degree of correlation will indicate that the rate of secretion is of considerable importance in relation to maximum production. Little hope of finding a close relationship between the type or conformation of the dairy cow and her production may be expected if the latter is true.

PERSISTENCY OF PRODUCTION

The declining segment of the lactation curve varies greatly in cows; some cows decline only slightly in production from month to month while others decline rapidly going dry at an

early date. In contrast to the physical characters of the dairy cow's anatomy which are associated with maximum secretion, is this intangible stimulus (similar to the possible stimulus for rapid secretion) persistency of secretion or the rate of decline of production during the lactation period. There is little in a cow's appearance to indicate whether she will decline rapidly in milk flow and soon become dry, or will decline very slowly in milk production and at the close of the normal lactation period still be producing a large flow of milk.

Possibly what the judge of cattle would call "dairy temperament" is the best external indication of the persistency of production of the cow. A lean, angular condition of flesh, however, may be a result of lack of feed, rather than due to the stimulation "to put everything into the milk pail." A cow which has been well fed and yet at the close of the lactation period is in low condition might be considered to be of the persistent type. In general, however, it may be said that the factor of persistency of production although of great importance in governing total yearly lactation milk yield, has no physical basis by which the extent of the stimulation may be determined.

The importance of persistency of production in relation to total production has not been generally realized. This has been due largely to the difficulty of securing a quantitative index of persistency. Recently the writer described a simple method of determining persistency from the ratio of the total yearly production to the maximum month's production (11). This ratio may be converted to a percentage basis indicating the average monthly decline in milk or fat production based on the preceding month's production. This gives a quantitative numerical expression for the important characteristic of milk secretion—the stimulation for persistent production.

In this paper especial attention will be given to the inheritance of the characters which are concerned with maximum production. The mode of inheritance of the stimulus for persistent production will be discussed in a paper to follow.

The change in production with age was corrected by the use of the conversion factors given in the Missouri Agricultural

Experiment Station Research Bulletin 79. As these factors were computed from the change in yearly production with age, there may be a slight error introduced as persistency of secretion changes slightly with ages. The error involved, however, is not sufficiently large to warrant redetermining this relation for maximum production and age.

COMPARISON OF GUERNSEY SIRES ON BASIS OF DAUGHTERS AVERAGE PRODUCTION DURING THE MAXIMUM MONTH

The purpose of the comparison of the sires on the basis of their daughters average production during the maximum month's production of the lactation period is designed to show the independence of the inheritance of the characters concerned with maximum production. Large production during the maximum month of the lactation period does not necessarily indicate high total production because of the factor of persistency. On the other hand, a combination of high maximum production associated with persistent production will always result in maximum yearly production.

In table 1 are listed the sires in order of the average fat production of their daughters during their maximum months production of fat compared with similar data for the dams of the daughters. This may be compared with the total yearly production of the dams and daughters. The persistency ratio (relation of total yearly fat production to the maximum month's fat production) is also given. The greater the numerical value of the ratio, the greater is the persistency indicated.[1] The differences in these two characters, maximum and persistent production, between the dams and daughters should be noted.

Might not the favorable combination of the genes concerned in the inheritance of the characters for maximum and persistent production in certain individuals causing greater producing ability than either family previously possessed, be produced in this .ay. While there is an occasional fortunate "nicking"

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[1] A complete discussion of the quantitative determination of persistency of production indicating the meaning of the numerical values of the ratios may be found in reference 11.

TABLE 1

Comparison of Guernsey sires based on the average mature equivalent fat production of daughters during the month of maximum production

Maximum of Daughters

NAME AND NUMBER OF SIRE	DAUGHTERS				DAMS			
	Number of yearly records, daughters	Average mature equivalent fat	Average mature equivalent, month of maximum	Total Maximum	Number of yearly records, dams	Average mature equivalent fat	Average mature equivalent, month of maximum	Total Maximum
King Masher 11048....................	23	665	75.0	8.739	16	593	62.9	9.585
King Bell 13482......................	10	707	75.0	9.501	8	581	59.8	9.908
Lavanton 11611.......................	10	729	74.6	9.873	10	690	73.8	9.375
Ne Plus Ultra 4th 29328..............	23	707	74.3	9.565	12	576	59.5	9.730
Bell-Founder 11681...................	19	694	70.7	9.776	10	643	66.6	9.763
King of Chilmark 20798...............	24	689	70.6	9.657	7	612	66.2	9.196
Langwater Stars and Stripes 21872....	14	708	70.5	10.185	10	559	56.2	9.875
Ne Plus Ultra 15265..................	46	687	70.3	9.755	32	569	59.7	9.551
Golden Secret of Lilyvale 10028......	23	674	70.3	9.645	16	592	64.6	9.201
Langwater Holliston 28055............	10	726	70.3	10.322	6	583	60.9	9.542
King of the May 9001.................	33	708	69.8	10.125	25	625	64.4	9.664
Mars of Woodcrest 9290...............	10	563	69.7	8.070	4	459	57.6	7.934
King's Vanguard 22719................	13	627	69.7	9.021	11	639	62.4	8.486
Florham Laddie 20431.................	30	662	69.6	9.314	11	592	62.5	9.492
Langwater Warrior 26509..............	23	701	69.3	10.044	18	764	76.7	9.941
Valentine Honour of the Passee G 3784	13	640	68.8	9.789	1	695	70.0	9.937
Lady Smith's Cherub 30760............	12	688	68.6	10.154	10	614	65.1	9.423
Brookmead's White Face 32211.........	17	716	67.7	10.814	13	499	53.1	9.465
Holden IV 12179......................	12	621	67.6	9.838	7	689	74.1	9.314
Joker of Riverside 21447.............	10	595	67.5	8.796	4	558	62.8	8.887
Ledyard Bay 11074....................	10	645	67.2	9.625	9	498	48.5	10.106
Langwater Hayes Rosie's King of the May 16723........................	27	681	67.0	10.135	22	602	64.1	9.382
Langwater Royal 14253................	27	616	66.8	9.107	22	554	62.2	8.889
Langwater Pencoyd 21830..............	18	658	66.8	9.837	16	679	69.4	9.757
Reputation of Portage 10695..........	11	632	66.6	9.642	5	584	68.2	8.973
Langwater Cavalier 21012.............	21	705	66.5	10.528	10	606	70.8	8.575
Ultra May King 27600.................	17	707	66.5	10.611	15	616	63.2	9.721
Charmante's Rose King 11746.........	11	624	66.0	9.442	7	632	57.4	9.334
Dairymaid's Criterion of Iowa 28187..	12	598	65.9	9.160	6	523	57.9	9.228
Demonstration of Roughwood 23133...	15	624	65.6	9.388	7	561	66.3	8.567
Gold Lassie's Jullian 27704..........	18	674	65.5	9.734	18	624	68.6	9.275

TABLE 1—*Continued*

NAME AND NUMBER OF SIRE	DAUGHTERS				DAMS			
	Number of yearly records, daughters	Average mature equivalent fat	Average mature equivalent, month of maximum	Total/Maximum	Number of yearly records, dams	Average mature equivalent fat	Average mature equivalent, month of maximum	Total/Maximum
Dolly Dimple's May King of Langwater 12997......................	10	630	65.3	9.630	9	626	63.1	9.682
Governor I. of the Chene 10563........	24	639	65.2	9.811	12	671	68.0	9.856
Glenwood's Main Stay 16th 9384.......	10	597	64.9	9.313	6	486	51.1	9.154
Jardiniere's Masher 20957.............	14	668	64.7	10.248	11	655	66.6	9.844
Masher's Galore 8572.................	22	615	64.4	9.551	15	470	50.9	9.320
Rosie's Golden King of Oakhurst 31630	10	639	64.4	10.208	9	615	60.1	10.248
Golden Noble II G1836..............	29	590	64.3	9.241	3	447	56.1	7.980
Lord Waukesha 10148................	18	575	64.3	9.056	14	522	59.1	8.881
Itchen Daisy's May King of Langwater 17349......................	24	637	64.2	9.829	18	530	56.0	9.486
Rex of Rich Neck 31472.............	15	645	64.1	10.119	13	669	65.2	10.241
Brockton of the Glen 15739..........	10	667	64.1	10.032	7	623	62.4	9.986
Golden Hero of L'Etiennerie 12647....	16	553	64.0	8.698	5	461	59.5	7.899
Jessy Rosie's Pride of Iowa 3955.......	19	519	63.9	9.056	14	504	53.9	9.319
Laverna's Ultra May King 24660......	12	585	63.8	9.667	7	565	65.6	9.463
Jethro's May King of Linda Vista 14591.............................	15	620	63.6	9.420	15	615	62.9	9.843
Rinaldo 8917........................	11	601	63.6	9.474	11	639	65.2	9.844
May King's Vrangu of Ingleside 15430	37	634	63.6	9.877	30	611	63.2	9.584
Yeoman's King of the May 17053......	84	610	63.5	9.487	64	508	58.3	8.850
Langwater Rival 14194................	16	639	63.3	9.988	10	596	60.4	9.874
Pencoyd's Golden May Secret 39626...	14	627	63.2	10.016	13	569	57.7	9.934
Jewel's Royal Combination of Wawa 15655.............................	11	589	63.2	9.374	9	493	53.1	9.413
Beda's May King 11893..............	41	640	63.1	9.950	31	592	60.3	9.748
Sailor Lad of the Fontaines 51090....	17	605	63.1	9.395	2	599	62.1	9.803
Honoria's Sequel II 40668...........	16	568	63.1	9.093	2	580	55.9	10.394
Victor of Pencoyd 18901..............	18	575	62.9	9.360	16	546	54.1	10.123
Pretoria's King of Midlothian 22641...	10	601	62.8	9.705	10	569	68.7	8.516
Bob Rilma's Monogram 29095........	10	608	62.8	9.653	10	523	59.5	8.872
Masher of Sarnia 19167..............	11	541	62.6	8.730	3	564	60.3	9.376
Hayes Cherub 2d 25147..............	16	630	62.5	10.047	9	595	65.2	9.371
Reservation Chesterfield 36609........	13	634	62.5	10.106	12	556	58.8	9.529
Don Diavola of Linda Vista 23565.....	11	635	62.5	9.878	8	584	59.5	9.808

TABLE 1—*Continued*

NAME AND NUMBER OF SIRE	DAUGHTERS				DAMS			
	Number of yearly records, daughters	Average mature equivalent fat	Average mature equivalent, month of maximum	Total Maximum	Number of yearly records, dams	Average mature equivalent fat	Average mature equivalent, month of maximum	Total Maximum
Langwater Peerless 19227.............	12	559	62.5	9.181	7	613	63.5	9.657
Cora's King of Bellevue 9779.........	11	569	62.5	9.114	3	494	83.6	7.804
Silver King of the Isle 14363.........	13	531	62.2	8.613	9	517	55.2	9.412
Spotswood Sequel 9686...............	30	532	62.1	9.160	17	502	55.5	9.133
Giltedge of Koshkonong Place 21989...	21	538	61.9	8.913	20	487	52.7	9.169
Onoko of Maple Row 11522...........	10	537	61.8	9.014	8	533	58.8	9.162
May King of Linda Vista 17946........	16	599	61.7	9.838	15	585	64.6	8.828
Border Raider 22243..................	30	618	61.6	9.980	28	566	69.7	9.483
Langwater Royal Master 23663........	14	632	61.5	10.254	9	626	65.0	9.565
May Rose King 2d 13130...............	16	594	61.5	9.963	9	582	64.2	9.052
Florham Monarch 20771...............	10	575	61.5	9.172	9	599	66.9	8.894
Modena's Yeoman of Langwater 10764	15	553	61.4	9.050	2	642	71.9	8.941
Jewel's Independence 10324..........	10	626	61.2	10.782	6	553	57.2	9.642
Langwater Traveler 38325............	15	678	61.0	8.676	11	529	65.3	10.009
Baubigny's Squire of Keewaydin 21834	11	577	60.9	9.476	10	578	68.8	8.408
Flora's Sequel of Vimiera 25905.......	20	572	60.8	9.397	1	526	60.7	8.662
Jethro Bass 11366....................	30	612	60.7	10.149	26	565	57.5	9.872
Sister's King 33653..................	11	562	60.7	9.236	4	423	52.9	8.195
Aimable of France 13739.............	10	552	60.7	9.076	5	496	54.6	8.762
Cherub's Winner 34180...............	14	575	60.6	9.417	11	491	59.0	8.324
Julian of Koshkonong Place 14409....	10	598	60.6	9.905	8	548	58.9	9.515
Langwater May King 13001...........	20	590	60.5	9.731	16	592	60.8	9.724
Moss Raider 22155...................	15	536	60.5	8.853	9	483	54.0	8.980
Penwyn of Rosendale 11282...........	11	566	60.4	9.404	8	683	69.5	9.945
France's Jewel's Champion 17970.....	13	554	60.4	9.751	1	513	50.5	10.140
Marshal of France 9051...............	12	549	60.4	9.206	5	506	53.4	9.560
Frank Rose 26342....................	11	562	60.3	9.332	9	483	51.9	9.328
Rockingham 18120....................	10	578	60.2	9.565	6	540	56.8	9.674
Gay Lad Du Braye G2026............	18	543	60.1	9.059	1	648	60.7	10.669
Florham Golden Lad 18119...........	12	571	60.1	9.525	7	747	71.1	10.496
Royal Governor of L'Etiennerie G1484	10	547	60.1	9.023	1	487	67.3	7.246
King of Medfield 15434................	10	549	59.9	9.153	4	431	52.0	8.200
Glenwood's Main Stay 40th 28108.....	12	536	59.9	9.509	3	523	63.7	8.245
Spotswood Rival 8346................	12	581	59.7	9.354	2	471	44.1	10.643
May King of Ingleside 12558.........	22	521	59.7	8.829	20	483	55.0	8.888

TABLE 1—*Continued*

NAME AND NUMBER OF SIRE	DAUGHTERS				DAMS			
	Number of yearly records, daughters	Average mature equivalent fat	Average mature equivalent, month of maximum	Total Maximum	Number of yearly records, dams	Average mature equivalent fat	Average mature equivalent, month of maximum	Total Maximum
Gay Boy of the Isle 16998	17	581	59.6	9.836	9	571	64.5	8.978
Casterilius of Nelsonville 18052	10	587	59.5	9.954	10	576	64.2	9.219
Don Bernando of Linda Vista 20617	11	629	59.3	10.660	7	527	56.6	9.430
May Rose King 8336	21	632	59.1	9.975	18	532	55.1	9.625
King Masher 8th 20973	11	595	59.1	10.046	9	537	56.5	9.519
Langwater Frederick 22268	17	553	59.0	9.076	12	592	61.4	9.694
Duke of Waumesit 13480	12	555	59.0	9.414	6	494	53.9	9.197
Bell Buoy of Linda Vista 19430	32	575	59.0	9.747	24	542	64.8	8.489
Glenwood's Reputation 7687	20	533	58.9	9.152	4	477	52.2	9.196
Langwater Golden Secret 26510	14	579	58.8	9.927	8	615	68.5	9.174
Buckthorn 4781	11	547	58.8	9.241	3	525	53.6	9.852
Marcia's Glenwood of Pinehurst 11560	10	545	58.7	9.365	6	514	54.8	9.480
Lord Mar 14359	30	558	58.6	9.543	5	669	81.9	8.184
Langwater Hambro 21011	27	551	58.5	9.605	23	482	53.1	9.226
Stranford's Glenwood of Pinehurst 13609	14	565	58.5	9.669	14	566	64.17	8.890
Beau Regal 13448	13	561	58.5	9.532	4	763	73.0	10.528
Ideal's Senator 14736	13	474	58.5	8.185	11	454	54.7	8.374
Starlight's Excelsior 7992	24	521	58.3	8.956	10	477	57.9	8.265
Pencoyd's Golden Secret 16550	14	538	58.3	9.474	10	612	63.7	9.668
Itchen May King of Stannox 34377	13	534	58.2	9.158	12	591	64.8	9.028
Robert's Criterion of Bellevue 26887	18	589	58.1	9.871	2	514	64.7	8.395
Triple Champion 13067	18	544	58.0	9.473	13	516	60.3	8.476
Dairymaid's Standard of Iowa 28946	12	541	58.0	8.816	11	533	58.2	9.272
Langwater Demonstrator 16451	66	611	57.9	9.865	46	515	55.4	9.392
Rutila's Gold Basis 5625	13	487	57.9	8.464	7	455	52.3	8.764
Begalore 10101	13	540	57.8	9.291	6	570	59.8	9.461
Langwater Frenchman 19226	28	537	57.7	9.172	24	542	58.6	9.261
Goldseeker of Anna Dean Farm 26106	18	547	57.7	9.479	10	726	70.6	10.047
Pretor 9316	14	524	57.6	9.211	4	519	56.1	9.198
Dimple Bloom 14369	10	557	57.4	9.637	2	584	60.1	9.679
Glenwood Boy of Haddon 4605	26	521	57.3	9.147	21	559	64.8	8.613
Barrington May King 19312	19	594	57.3	10.376	17	570	51.7	9.982
France's Masher 2d 7248	12	512	57.3	8.954	1	649	68.2	9.511

TABLE 1—*Continued*

NAME AND NUMBER OF SIRE	DAUGHTERS				DAMS			
	Number of yearly records, daughters	Average mature equivalent fat	Average mature equivalent, month of maximum	Total Maximum	Number of yearly records, dams	Average mature equivalent fat	Average mature equivalent, month of maximum	Total Maximum
Moonlight King of Anna Dean Farm 29586	13	543	57.2	9.615	5	438	57.8	7.325
Lehigh's Golden Emperor 15761	18	516	57.2	8.999	6	458	60.4	7.616
Endymion 8916	16	533	57.0	9.283	8	568	59.3	9.550
Sequel's Monogram 15649	30	543	56.9	9.526	11	460	56.2	8.878
Itchen May King 25174	20	574	56.9	10.108	17	599	63.5	9.484
Langwater Dictator 15668	11	513	56.9	9.062	9	421	49.6	8.387
Guiding Star 12423	15	486	56.9	8.632	11	579	65.1	8.914
Langwater Royal 7th 20632	18	588	56.9	9.976	6	455	54.6	8.629
Jury of Koshkonong Place 16793	24	497	56.8	8.833	16	451	49.4	9.180
May Rose Secret of Pencoyd 27844	14	549	56.6	9.668	9	593	62.0	9.656
Governor of the Vanquiedor 39925	20	508	56.6	9.284	2	483	60.1	8.036
Golden Secret 12599	15	529	56.5	9.218	7	507	57.5	8.978
Ivy's Emblem G3804	10	566	56.5	9.864	1	662	70.1	9.435
Galaxy's Sequel 16904	53	523	56.4	9.354	13	542	60.5	9.001
Raymond's Pioneer of Lewistown 19103	20	590	56.3	9.351	14	617	66.5	9.243
Rutila's Sheet Anchor 5701	11	505	56.2	8.984	4	472	51.8	9.046
Robina's Standard 7254	21	473	56.1	8.515	11	535	59.7	9.065
Golden Secret of Pencoyd 23462	22	536	56.1	9.561	22	587	58.1	9.924
Pride of Day 17126	14	529	56.0	9.515	8	540	59.0	9.142
General Bay 16177	11	506	56.0	9.372	10	621	65.0	9.708
Alderney Raymond 26357	29	516	56.0	9.244	2	640	64.8	9.891
Pencoyd Quaker Boy 35552	10	556	55.9	9.562	2	559	57.0	9.807
Yeoman's King Victor 22295	15	539	55.8	9.837	15	576	62.3	9.295
Governor of the Chene G1297	112	524	55.7	9.500	2	545	59.2	9.123
Spotswood Masher Sequel 9687	11	504	55.7	9.017	7	444	54.0	8.355
Uncle Jim 16740	17	540	55.7	9.896	15	574	61.2	9.242
Dean of the May 21815	20	526	55.7	9.135	14	547	57.8	9.556
Hope's May Rose of Maple Hill 35903	12	518	55.6	9.329	7	542	57.8	7.795
Langwater Islander 31329	10	530	55.6	9.658	7	502	57.5	8.732
Langwater Fisherman 21873	12	549	55.4	10.053	6	512	53.8	9.604
Princesse's Jewel 24877	31	515	55.3	9.343	3	550	57.1	9.652
Prince of Sarnia 22000	21	523	55.3	9.621	9	492	50.6	9.763
Beda's May Day 34995	10	546	55.3	8.946	7	593	68.2	8.702

TABLE 1—*Continued*

NAME AND NUMBER OF SIRE	Number of yearly records, daughters	Average mature equivalent fat	Average mature equivalent, month of maximum	Total / Maximum	Number of yearly records, dams	Average mature equivalent fat	Average mature equivalent, month of maximum	Total / Maximum
		DAUGHTERS				DAMS		
Langwater Raritan 17052.............	17	566	55.2	9.632	17	499	52.7	9.423
Duke of Mouilpied G2045.............	11	523	55.2	9.450	1	408	48.8	8.340
Alderney II G2215....................	19	582	55.1	9.871	1	464	52.3	8.863
Jacqueminot of Linda Vista 23564....	20	537	55.1	9.840	12	522	55.2	9.544
Masher's Sequel 11462.....	70	511	55.0	9.600	17	501	58.4	8.667
Glenwood's Stranford 9386...........	20	516	55.0	9.349	14	448	48.7	9.270
Robert's Boy 21662......	13	520	55.0	9.277	3	568	64.0	8.710
Auricula's Main Sheet 8870..........	10	495	55.0	9.099	5	528	53.5	9.821
Glenwood's Combination 8th 12550...	11	504	54.8	9.205	5	438	46.4	9.510
The Conqueror II 15323.............	17	542	54.7	9.929	4	504	53.8	9.276
Wolfram 5640.......................	10	470	54.7	8.670	5	667	67.1	9.948
Lily Ella's Squire 6597.............	15	494	54.5	9.144	5	456	46.1	10.219
Selma's Main Stay's Son 23585.......	14	476	54.5	8.500	9	520	64.7	8.072
George Washington of Fairfield Farm 10866.............................	12	516	54.5	9.459	3	548	60.8	9.051
Raymond of the Preel IV 19235.......	17	505	54.4	9.445	3	399	44.3	8.984
Itchen Red Raider 27342............	10	549	54.3	10.517	8	634	62.7	9.909
Governor of the Cateret G3421.......	13	570	54.2	9.849	2	597	57.0	10.453
Allenwood King Regent 23611........	13	562	54.2	10.354	13	508	52.9	9.697
Raymond of the Preel VI 14360.......	12	467	54.1	8.640	3	494	52.4	9.440
Dairymaid's Pride of Iowa 14941......	12	518	53.9	9.596	5	601	68.7	9.095
Selma's Glenwood 12596.............	19	478	53.8	8.937	15	526	60.7	8.809
Souvenir of L'Etiennerie 21925.......	17	523	53.8	9.747	6	584	63.7	9.124
St. Austell Dreadnaught 34671.......	10	501	53.8	9.429	9	502	54.4	8.814
Skeezicks 9979......................	13	483	53.7	9.113	9	537	65.3	8.148
Sir Snowdoun 19252.................	20	496	53.5	9.104	17	431	47.6	9.094
Radium 9193.......................	12	445	53.5	8.425	9	495	63.8	7.930
Frank Rilma 21901..................	11	520	53.5	9.732	8	485	50.2	9.794
Dairymaid's King 12898..............	21	490	53.4	9.200	18	438	54.8	8.113
Nelson II A52......................	12	497	53.4	9.452	1	476	54.8	8.861
Lord Mar V 18961..................	10	456	53.4	8.890	1	408	42.5	9.589
Strong Anchor 5849.................	15	491	53.2	9.296	3	441	45.8	9.608
Glenwood's Main Stay 6067...........	26	482	53.0	9.290	16	440	49.0	9.077
Lohier's Beta Raider of Waddington 35442.............................	20	516	52.8	9.731	12	496	63.5	8.017

TABLE 1—*Concluded*

NAME AND NUMBER OF SIRE	DAUGHTERS				DAMS			
	Number of yearly records, daughters	Average mature equivalent fat	Average mature equivalent, month of maximum	Total Maximum	Number of yearly records, dams	Average mature equivalent fat	Average mature equivalent, month of maximum	Total Maximum
Dandy of Beirnetown 29963...........	17	523	52.7	9.742	16	512	48.2	9.685
Northern Boy G1779.................	11	448	52.7	9.268	1	621	72.5	8.558
Norfolk Squire 11565.................	14	504	52.6	9.584	6	506	56.1	9.055
Raymond of Blaye Farm A191........	13	521	52.6	9.665	1	483	54.8	8.798
Dan Patch of the Isle 21293..........	11	517	52.6	9.850	11	511	65.6	9.251
Rhea's King of the May 14368........	21	514	52.4	9.941	15	511	57.7	8.869
Barmouth of Pencoyd 11059..........	12	461	52.0	8.954	6	564	64.0	7.971
Glenwood's Son of Gayhead 19617....	10	551	51.9	10.210	3	554	60.3	9.173
Christy of Pinehurst 31st 31984.......	10	479	51.6	9.177	5	419	48.8	8.577
Raymond of the Preel 11353..........	17	451	51.5	8.950	4	476	54.7	8.733
Christie's Combination 14651.........	10	475	51.2	9.389	9	427	46.2	9.370
Gypson's Count 8125.................	18	434	51.0	8.569	1	416	51.7	8.039
Roy of Norwood 8141.................	18	530	50.7	9.344	8	470	51.7	9.057
Raymond of the Preel II 13381.......	12	475	50.3	9.561	7	538	58.2	9.145
Fanny's Sequel 19563.................	28	478	49.9	9.428	7	574	61.1	9.459
Penwyn 2d 7559......................	10	400	49.5	8.213	5	428	58.1	7.462
Governor of the Gree 19123..........	15	500	49.3	10.150	2	519	58.2	8.908
Rouge II's Son 18587.................	10	471	49.3	9.592	9	462	53.6	8.756
Golden Anne's Fernwood of Homestead 11916........................	16	461	49.1	9.314	2	519	50.0	10.268
Malcolm of Maplehurst 5626..........	12	454	48.7	9.412	7	512	50.9	10.035
Capt. Robbie 7146....................	11	442	47.4	9.435	10	484	53.4	9.212
Preel VII's Champion Butter Boy 26132.............................	13	425	46.2	8.960	12	448	47.2	9.573
Galaxy's Lavinius 12548..............	20	435	45.6	9.616	17	463	53.4	8.732
Ukiah 14344.........................	14	405	45.1	9.174	14	447	48.5	9.282
Overture of Prospect 32821...........	10	456	45.1	10.067	6	395	41.6	9.686
Sunburst's King Maiden 37423........	16	420	44.9	9.305	12	421	46.3	8.950
Coralette's Son 3987.................	12	399	44.9	8.976	11	421	51.9	8.167
Vaillant Coeur 7749..................	13	416	41.5	10.038	10	441	50.3	9.966

with resultant improvement, a much more certain plan of securing cows which possess these two characteristics to a high degree would be the use of sires having already demonstrated

through their daughters high maximum and persistent production. The next best plan would be the use of sons of such sires.

COMPARISON OF THE MAXIMUM PRODUCTION OF THE DAMS AND DAUGHTERS

As in the study of total yearly fat production, the relative effect of the sire and the dam on the daughters maximum production is of great interest. A summary of the results of the

TABLE 2

Dam and daughter comparison of Guernsey sires

DAM'S PRODUCTION OF FAT MAXIMUM MONTH (CLASS)	SIRE'S CLASS: DAUGHTER WITH MAXIMUM MONTHLY FAT 40 TO 44 POUNDS		SIRE'S CLASS: DAUGHTER WITH MAXIMUM MONTHLY FAT BETWEEN 45 AND 49 POUNDS		SIRE'S CLASS: DAUGHTER WITH MAXIMUM MONTHLY FAT BETWEEN 50 AND 54 POUNDS		SIRE'S CLASS DAUGHTERS MAXIMUM MONTHLY FAT BETWEEN 55 AND 59 POUNDS		SIRE'S CLASS DAUGHTERS MAXIMUM MONTHLY FAT BETWEEN 60 AND 64 POUNDS		SIRE'S CLASS DAUGHTERS MAXIMUM MONTHLY FAT BETWEEN 65 AND 69 POUNDS		SIRE'S CLASS DAUGHTERS MAXIMUM MONTHLY FAT BETWEEN 70 AND 75 POUNDS	
	Pairs	Daughters, average fat	Pairs	Daughters, average fat	Pairs	Daughters, average fat	Pairs	Daughters, average fat	Pairs	Daughters, average fat	Pairs	Daughters, average fat	Pairs	Daughters, average fat
		pounds		*pounds*		*pounds*		*pounds*		*pounds*		*pounds*		*pounds*
30–39	4	45.2	5	42.6	10	46.9	22	55.1	10	55.9	1	58.4	4	74.4
40–49	11	45.0	41	45.5	77	52.0	153	55.9	123	59.9	33	62.8	10	68.6
50–59	14	43.5	25	48.1	98	53.6	227	57.6	178	64.1	72	66.0	28	69.8
60–69	2	47.0	13	49.1	43	53.5	144	60.1	151	64.0	92	70.4	16	72.9
70–79			4	58.1	40	56.4	69	60.8	80	64.8	41	69.6	15	79.1
80–89					10	56.7	13	60.0	18	66.2	23	71.7	5	79.5
90–99					2	61.1	4	64.3	7	66.2	3	78.7	2	76.2
100–109					3	57.4	2	60.1	1	71.5	5	74.6		
110–119					—	—								
120–129					1	64.5								

dam and daughter comparison is shown in table 2. It will be noted that the sires are grouped into classes on the basis of their daughters' average maximum months' fat production. Then the average production of daughters by dams of varying productivity was determined. In order to determine the average influence of the dam on the daughters production, straight line equations most nearly fitting the observed values were calculated.

The equations are of the form $D = a + b\,d$ in which D is the average production of the daughters; d the production of the dam, b the constant increase in production of the daughters for each pound of increase in the production of the dams, and a the sire's potential transmitting ability with dams of such low production that they contribute nothing to the daughters.

CLASS OF SIRES (SIRE'S DAUGHTER'S MAXIMUM PRODUCTION)	CONSTANTS	
	a	b
70 to 75 pounds fat.............................	52.2	0.34
65 to 69 pounds fat.............................	52.1	0.25
60 to 64 pounds fat.............................	52.4	0.17
55 to 59 pounds fat.............................	50.1	0.14
50 to 54 pounds fat.............................	43.8	0.16
45 to 49 pounds fat.............................	36.2	0.21
40 to 44 pounds fat.........	38.3	0.15

The value obtained for b in each case indicates the contribution of the dams to the daughters inherited producing ability during the maximum month. The dam's contribution was found to vary from 15 to 34 per cent, being greatest in the groups of sires with daughters having the highest maximum. The average influence of the dams for all groups was 20 per cent. These results may be expressed in the form of the following equation.

$$\text{(1)}\quad \begin{matrix}\text{Daughter's maximum}\\ \text{fat production}\end{matrix} = 0.20 \times \begin{matrix}\text{dam's maximum}\\ \text{fat production}\end{matrix} + \begin{matrix}\text{sire's potential}\\ \text{transmitting}\\ \text{ability}\end{matrix}$$

$$\text{(2)}\quad \begin{matrix}\text{Sire's potential}\\ \text{transmitting ability}\end{matrix} = \begin{matrix}\text{daughter's maximum}\\ \text{fat production}\end{matrix} - 0.20 \times \begin{matrix}\text{dam's}\\ \text{maximum}\\ \text{fat production}\end{matrix}$$

These equations indicate that when using the dam's producing ability as an index of her transmitting ability and using a sire's daughter's average mature equivalent fat production as his index of transmitting ability that the former can be given only 20 per cent consideration. The second equation will be useful in determining the sire's potential transmitting ability when the daughter's and dam's production is known. In comparison

to the equations indicating the relation between dams, daughters and sires, it will be noted that the dam apparently contributes more to maximum production than to yearly production.

THEORETICAL DISCUSSION OF RESULTS

Can these results which are far from the results one might expect be reconciled with genetic and cytological facts? The following discussion is an attempt at such an explanation. If it is incomplete or faulty it will at least be a starting point for further experiments and discussion which in the end will result in an advance in our knowledge of the inheritance of the factors concerned in milk secretion.

The cytological facts in regard to the number of chromosomes carried by the egg and sperm in dairy cattle has been worked out by Wodsedalek (12). He has found that the female is the result of the union of an egg containing 19 chromosomes and a sperm containing 19 chromosomes. The male is the result of a union of a sperm containing 18 chromosomes and an egg containing 19 chromosomes. In other words, the female is homozygous and the male heterozygous for the sex chromosome. If the genes for large milk and fat secretion were carried on the sex chromosome (were sex-linked) it would be the female which would have the greater possibility in the transmission of the genes which influence milk and fat secretion. As it has already been shown that the sire can transmit his producing ability through his sons to his granddaughters to a high degree, even when he does not furnish a sex chromosome to his son, it is apparent that the genes concerned in the transmission of the characters for milk secretion do not lie on the sex chromosome and, therefore, are not sex-linked.

Due to the fact that the sire and dam contribute the same number of chromosomes to the female offspring, it has been assumed that the production of the daughters would be intermediate between the parents. This indeed has been found true for many characters which have been studied. In all cases studied, however, both the male and female exhibit the characters concerned. Milk secretion differing from such characters as size, etc., is

expressed only in the female sex.   No direct measure of the sire's inheritance is possible.

While the female's production of milk and fat has been taken not only as the measure of her inheritance, but also of her transmitting ability, the male's measure of inheritance has been solely one of transmitting ability.   In other words, an individual production test has been compared with a transmission or progeny performance test.   It is this difference which has caused the sires to appear to contribute more to their daughers' producing ability than do the dams.

Why is a production test of the dam inferior to a progeny production test.   The answer to that question can be found in Mendel's Law of inheritance.   It has been found that plants and animals do not always breed on as they appear.   Only the recessives always breed true to their appearance.   The dominants are of two kinds, pure dominants and heterozygous dominants, only the former of the two kinds breeding true although in appearance are exactly similar to those heterozygous.

The theory is advanced that the factors which are concerned with the inheritance of large milk and fat secretion are in part at least dominant over the factors which produce a condition of limited secretion.   The fact that dairy cattle do not breed true to their performance indicates that some, if not all, of the factors concerned in high production are dominants.   The low producers maintain from generation to generation a yield of milk only sufficient to raise their calves.

Assuming that a large proportion of the factors for large production are dominant the experimental results obtained are easily explained.   As already mentioned, it is believed that a large number of genes or factors are concerned in bringing about maximum secretion.   The number and the relative contribution is a problem of the future.   As it is difficult to deal with large numbers of factors, a trihybrid will be used as an illustration. Let us assume further that the dominants have a value of 30 pounds of fat and the recessives 5 pounds of fat, then:

Cow I of the composition AABBCC would produce 90 pounds of fat during the maximum month.

Cow II of the composition AaBbCc would produce 90 pounds of fat during
   the maximum month.
In segregation cow I would produce eggs of the following kinds:
   ABC   ABC   ABC   Average 90.
    90   90   90
Cow II would produce eggs of the following kinds:
   ABC   ABc   AbC   Abc   aBC   aBc   abC   abc Average 52.5.
    90   65   65   40   65   40   40   15

Cow I being homozygous for 90 pounds of fat during her maxi-
mum month would contribute 90 pounds to all her progeny.
Cow II being heterozygous although producing 90 pounds of
fat would transmit only an average of 52.5 pounds of fat if all
combinations were represented in her progeny. A single daugh-
ter might go as low as 15 pounds of fat or as high as 90 pounds of
fat depending on the particular combination of genes which
were received—the sires contribution not being considered.

The actual experimental results obtained indicate a high aver-
age degree of heterzygosity on the part of the females of the
breed for the closer the approach to homozygosity the closer
will the production records indicate the transmitting ability.
This may be explanation why the dams maximum fat produc-
tion is closer to her transmitting ability than her yearly record
is an index of transmitting ability for yearly production.

The above illustration if applied to a sire indicates the value
of a progeny performance test. Instead of using a single pro-
duction test, the sire's ability is based on a group of daughters
which tends to include all possible combinations of genes in
segregation.

SUMMARY

1. Rather than to attempt a factorial analysis of the milk
secreting function of the dairy cow, it is proposed that the charac-
teristics of the lactation curve be studied in order to group to-
gether all factors which may affect certain parts and to study
these characteristics from a genetic standpoint.

2. The two most important characteristics of the lactation
curve of milk secretion are believed to be the height of pro-
duction of milk and fat during the maximum month or other

convenient unit of time, and the persistency of secretion or the rate of decline of production.

3. A study was made of Guernsey Advanced Registry records to determine the relative effect of the sire and dam on the daughters maximum fat production.

4. The results were expressed by the two following equations:

$$(1)\ \text{Daughter's maximum fat production} = 0.20 \times \text{dam's maximum fat production} + \text{sire's potential transmitting ability}$$

$$(2)\ \text{Sire's potential transmitting ability} = \text{daughter's maximum fat production} - 0.20 \times \text{dam's maximum fat production}$$

5. The cytological studies indicate that the sire and dam contribute the same number of chromosomes to the female. The male is the result of the usual number of chromosomes from the dam and one less from the sire. This is taken to indicate that the above results can not be explained by sex-linked inheritance.

6. The results are believed to be explained by assuming that the majority of the genes concerned in the inheritance of large fat secretion are dominant over small secretion. Such being the case, the only true test of an animal's transmitting ability is a test of the progeny as compared to a performance record.

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SWEETENED CONDENSED MILK

VI. TALLOWINESS*

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There are, perhaps, more different and distinct flavors produced from the decomposition of milk fat than from any other fat or oil. While in other industries any abnormal flavor of fats is denoted as "rancidity," in the milk products industries attempts are made, particularly in recent years to distinguish between several different flavors, such as tallowiness, fishiness, rancidity, metallic and so on.

Tallowiness is a flavor remotely resembling tallow. It has been recognized in butter, powdered whole milk and raw milk. It is commonly accompanied by bleaching of the fat, and is generally attributed to the action of oxygen on the fat, the reaction being catalysed by light, ultraviolet light, metals, or free fatty acids (1). Mention was made of this defect in condensed milk in one of our previous papers (2), at which time it was pointed out that the flavor had been found to develop when unusual amounts of copper were present. The purpose of this paper is to present data in support of that statement, to contribute other information concerning the cause of this defect, and to discuss methods of prevention.

The flavor of tallowy condensed milk resembles to a considerable degree tallowy butter and powdered whole milk. It is a condition not so marked as that of rancidity or bacterial thickening. The acidity does not increase, neither does the viscosity. The odor and taste except in rare instances are very weak. Unless it is an extremely bad case the product may be used in baking and in candy making. In hot beverages the flavor is brought out to such an extent as to make it unusable. On opening a tin

the sample appears sometimes but not always a little paler than normal. The tallowy flavor of the freshly opened sample remains even after exposure to the air for several days.

Since it has been generally believed that tallowiness in other milk products is due to the oxidation of fat, a catalyst possibly being necessary for the reaction to take place, it seems fair to postulate that a similar reaction is involved in this case. Tins of condensed milk always include some air, and the product may contain a great deal of copper since so much of the condensing equipment is ordinarily constructed of that material. Light is eliminated as a factor here, since commercial samples are found only in tin; particular care was taken in this experimental work that samples in glass were kept in a dark place.

Oxygen as a factor in tallowiness production

It has often been noted in commercial samples, though this is not always the case, that it is only the layer of condensed milk at the top and exposed to the air space which is found tallowy. And this has been always the rule in experimental samples, unless care was taken to mix the liquid from time to time, in this way bringing fresh surfaces in contact with the air space. It has always been found in experimental samples that when tins or bottles were filled entirely full, there was less tallowiness, if any at all, than when a liberal air space remained above the liquid.

Experiment 1. To a sample of condensed whole milk was added 50 mgm. of copper in the form of copper lactate. The weighed amount of the salt was dissolved in a few cubic centimeter of water, added to the condensed milk, warmed to facilitate mixing, and stirred well with an egg-beater. This was divided into three parts each placed in a fruit jar so that the jar was only about three-fourths full. The air was displaced with oxygen gas in sample 1, and with carbon dioxide in sample 2; air filled the space in sample 3. The jars were well sealed to prevent escape of gas, and held in an ice storage room for later inspection.

Inspections for flavor were made at the end of twenty and forty days: At each time sample 2 was unchanged in flavor. Sample 1 was described as very tallowy at twenty days and extremely tallowy at forty days. Sample 3 was distinctly tallowy on both occasions but never becoming as strong as sample 1.

This experiment shows that oxygen is a necessary factor for the production of tallowiness in sweetened condensed milk.

Production of tallowiness a chemical not a biological change

In all the experiments here described the samples were held in an ice storage room until time of inspection. This procedure was adopted after several unsuccessful attempts to prevent growth of mold in other ways, it being impossible in the preparation of samples to prevent contamination. Due to the fact that condensed milk ordinarily becomes tallowy only after weeks or months, there is sufficient time for mold to develop and make it impossible to inspect the sample for tallowy flavor. It was found that the temperature of the ice storage room (27° to 32°F.) was such that mold growth was prevented while tallowiness developed just as rapidly as at room temperature. This is an indication that the reaction is due to the chemical oxidation of fat, and not to organisms or enzymes.

However, as was pointed out in the preceding paper of this series (3), it is possible that free fatty acids may catalyze the reaction involved in tallowiness formation. Fatty acids in milk products are derived most commonly from an enzyme hydrolysis of butter fat. In this way, therefore, enzymes may indirectly hasten tallowiness. This point of view might explain the findings of Palmer and Combs (4) where experiments bore indication that in butter a greater degree of tallowiness results when enzymes are present.

Other proofs of the statement that biological processes have nothing to do directly with tallowiness in condensed milk are the following:

1. Exposure of the product to very high temperatures for long periods of time did not lessen the effect, in fact it seemed that

when samples were exposed to a great deal of heat more tallowiness developed.

2. Bacterial counts on eight samples of tallowy condensed milk were—175, 220, 550, 770, 1050, 2570, 1690, 1400; the two which are lowest in bacterial counts happened to be strongest in the tallowy flavor.

3. At one time some samples to which had been added some copper for the purpose of obtaining tallowiness were divided and to one series was added 0.1 per cent mercuric chloride, to the other none. Later inspection showed that both series of samples became tallowy at equal rates.

TABLE 1

SAMPLE NUMBER	INSPECTION AT THE END OF			
	25 days	45 days	70 days	100 days
1	0	0	0	0
2	0	0	+	++
3	0	+	++	+++
4	++	+++	+++	+++
5	+++	+++	+++	+++
6	+++	+++	+++	+++
7	+++	+++		
8	0	0	+	++

Normal flavor, 0; tallowiness, distinct, +; tallowiness, fairly strong, ++; tallowiness, exceedingly strong, +++; blank spaces indicate that no inspection was made.

Copper as a factor in tallowiness production

Experiment 2. A quantity of freshly condensed milk was divided and treated as follows: Sample 1, no addition; sample 2, adding per kilo of product 2.5 mgm. of copper in the form of lactate; sample 3, 5 mgm.; sample 4, 10 mgm.; sample 5, 25 mgm.; sample 6, 50 mgm.; sample 7, 25 mgm. of copper as ammoniacal cupric oxide; sample 8, clean bright copper strips were introduced so that one end extended above the surface of the milk.

Several samples of each series were placed in sterile bottles,

leaving some air space. At each inspection a sample was used that had not been previously opened. Particular care was taken each time that the inspector did not know to which series the sample under consideration belonged until after judgment had been passed. Table 1 gives the results of inspection for tallowy flavor.

The speed of tallowiness production and the degree of flavor is seen to increase with the amount of copper present, an addition of as little as 2.5 mgm. per kilo being effective. In experiment 1 it was shown that oxygen is a necessary factor. It is concluded, therefore, that tallowiness is here caused by the action of oxygen on some constituent of the milk, the reaction being hastened by the presence of copper. (But this is not to exclude the possibility that other catalysts than copper may be effective.)

Five batches of condensed milk which were found to show varying degrees of tallowiness under most conditions were found to contain 3.9, 4.2, 5.7, 7.7, and 11 mgm. copper per kilo. Rice and Miscall (2) have reported finding in normal canned milk 2.4 to 4.8 mgm. copper per kilo with an average of 3.7 mgm. It would seem, therefore, that some batches of normal condensed milk might contain sufficient copper to cause tallowiness under suitable conditions, and this has been found to be the case. On several occasions condensed milk as canned in the factory and placed on the market did not become tallowy, while portions of the same batch held in fruit jars, leaving a liberal air space, did develop the flavor. Also in a few instances portions of condensed milk to which an excess of copper was added were filled into containers so that practically no air space remained, it was always found that tallowiness developed to a lesser degree than when more air remained in contact with the surface of the liquid. The conclusion to be reached from these experiments is that either oxygen or copper may may prove to be the limiting or controlling factor in tallowiness production.

Experiment 2 shows also that copper in other forms than lactate is effective. The metal in contact with condensed milk produces the tallowy flavor but slowly, due no doubt to the slowness with which it dissolves and diffuses in the milk. In another

experiment it was found that copper chloride and copper oxide caused tallowiness. The active factor is no doubt, therefore, the cation of these salts.

Tallowiness due to a decomposition of the fat

Since tallowiness has always been found in butter and separated butter fat as well as other fats, and since it has been noted in powdered whole milk but not in powdered skim, there seems little doubt that in those products the abnormal flavor is caused by a chemical change in the fat.

An attempt was made to obtain normal condensed skimmilk with no fat, but the nearest approach to it was a sample with 1.39

TABLE 2

SAMPLE	INSPECTION AT END OF	
	12 days	30 days
Skim, 40 mgm. copper per kilo......................	+	+
Skim, 20 mgm. copper per kilo......................	+	+
Skim, 0 mgm. copper per kilo......................	0	0
Whole, 40 mgm. copper per kilo....................	++++	++++
Whole, 20 mgm. copper per kilo....................	++	++
Whole, 0 mgm. copper per kilo....................	0	0

See table 1 for key to symbols.

per cent fat. This was compared with normal condensed whole milk with 8 per cent fat: To each was added 20 and 40 mgm. of copper in the form of lactate. The various portions were placed in fruit jars filling them about three-fourths full; the remaining space contained oxygen gas. Results of inspections are given in table 2.

Two other experiments gave results similar to those here shown. Condensed skimmilk may become slightly tallowy but to a very much less degree than condensed whole milk under the same conditions. Since, besides the fat, all other milk constituents are present in skimmilk in higher percentages than in whole milk, there is no doubt that tallowiness here is due to changes in the fat.

Exposure of condensed milk to air at the time of condensation

Since the action of air or oxygen with a catalyst will produce tallowiness during storage, it seemed possible that exposure to air during condensation might cause the same flavor defect, the catalyst not being necessary. Vacuum pans leak at the bottom quite frequently, thus permitting a flow of air through the milk during condensation.

For experimentation on this point provision was made for condensing in glass. A 3-liter flask was used in connection with a vacuum pump so that boiling would take place at 130° to 135°F. Batches containing 4 pounds of milk with the proper proportion of sugar could be thus handled.

Check batches prepared in this apparatus never became tallowy but when copper salts were present during condensation this flavor always developed.

A batch run while air was continually bubbled through the milk did not become tallowy. On other occasions portions of normal factory condensed milk were warmed to 135°F. and air bubbled through for one-half hour, and in other portions oxygen was whipped in with an egg beater. Tallowiness was not produced by these methods.

It is to be concluded that this flavor is not caused from the exposure of a batch to air or oxygen at the time of condensation.

Condition of factory equipment and its relation to tallowiness production

It has frequently been observed that after a vacuum pan has not been used for a considerable period of time the first batch run is almost sure to become tallowy. Analyses have shown also that the percentage of copper is unusually high in such a batch: One day's run in a factory on a pan which had not been previously used for several months produced condensed milk which when canned regularly into tins became strongly tallowy in thirty days. There was found 7.7 mgm. copper per kilo.

A 100-pound batch ran on a small (copper) experimental vacuum pan not used for two weeks previously became strongly tallowy, and was found to contain 11 mgm. copper per kilo.

It is common knowledge that the surface of the copper of unused pans and hot wells becomes covered with a black oxide coat, but when this equipment is in constant use the surfaces remain bright. Rice and Miscall (2) have shown that more copper is dissolved in milk from an oxidized copper surface than from one that is clean and bright. It must be concluded, therefore, that tallowiness in batches manufactured in equipment not previously used for some time is caused by the excessive amounts of copper dissolved from the surfaces.

Observations on some experimental batches prepared in glass with and without the addition of a piece of copper gauze fully corroborate these conclusions.

Effect of other metals on tallowiness

Aside from copper practically the only metals ordinarily coming in contact with milk in condensing equipment are tin and iron.

Experiments carried out with these metals in the same way that has been described with copper showed that tin does not catalyze the reaction at all. The action of iron was generally found positive but weak: For instance, in one experiment 25 mgm. per kilo of iron in the form of tartrate was added to a quantity of normal condensed milk, when frequent inspection showed that faint tallowiness developed only at one hundred twenty-five days. Under the same conditions 25 mgm. of copper produce extreme tallowiness in less than twenty-five days.

Tin and iron cannot be important in the production of the tallowy flavor in condensed milk.

SUMMARY

1. Tallowy condensed milk is produced through the action of the oxygen of the air in the container on the butter fat of the milk, the reaction being catalyzed ordinarily by small amounts of copper.

2. Tallowiness develops below 32°F. apparently just as rapidly as at room temperature; heat sterilization of the product does not prevent it; the bacterial counts of tallowy samples are low; addition of strong preservatives do not prevent the development

of the flavor. All these points are taken as proof that the reaction involved is chemical and that biological agencies have nothing to do with it.

3. Pure oxygen was found to be more active in producing tallowiness than air. The development of the flavor could be prevented by displacing the air with carbon dioxide, or, by filling containers so full that no air space remained. Tallowiness increases, therefore, with the extent of exposure to oxygen.

4. Tallowiness was found to increase with the amount of copper present. When the percentage of copper is small, much more oxygen or air space must be in contact with the sample to produce the flavor. And when there is but little air space more copper must be present.

5. Condensed skimmilk may become slightly tallowy if it contains any fat at all, which it usually does in commercial practice.

6. Copper condensing pans and hot wells that have not been used for some time previously yield an excessive amount of copper to the first few batches manufactured; accordingly, these batches are most likely to become tallowy.

7. Tin was found not to catalyze the tallowiness reaction. Iron was found to do so but to a very much less degree than copper; it cannot be important in practice.

Practical conclusions

1. To prevent tallowiness the air space in the cans and the copper in the milk should be reduced to a minimum.

2. Since open pasteurizers, holding tanks and hot wells may, if made of copper, yield more copper to the milk than vacuum pans (2), particular care should be taken that the milk does not remain in any of these articles of equipment longer than necessary. Wherever possible all copper equipment should be replaced by other materials for the manufacture of sweetened condensed whole milk.

3. In experimental and laboratory samples tallowiness developed only at the surface of the milk in contact with the air, but in samples obtained on the market this flavor may be found throughout the can. This is attributed to the fact that in ship-

ping and handling, the contents of the cans are so mixed that a new surface is frequently brought into contact with the air.

4. After a copper pan or hot well has not been used for a long time, particular care should be taken to clean the surface so that a minimum of copper will dissolve in the first batch. In the experimental work no method of cleaning was found which would entirely prevent tallowiness from developing in the first few batches run. It is therefore concluded that such batches should be put on a quick market or used in ice cream or candy manufacture.

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FACTORS FOR ADJUSTING MILK AND BUTTERFAT RECORDS OF REGISTER OF MERIT JERSEY COWS TO A UNIFORM AGE BASIS*

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The influence of age on the production of milk and butterfat by dairy cows is not a new problem, and dairy literature contains much information on the subject. In 1917 (1) Pearl and Patterson reported the results of an analysis of seven-day Jersey milk records taken from "Jersey Sires and Their Tested Daughters," a publication issued by the American Jersey Cattle Club in 1909. One of the conclusions of these authors was that maximum production is reached at approximately the age of 8 years and 7 months. In 1919, Pearl, Gowen, and Miner (2) studied 2153 yearly milk records from the first two volumes of the Register of Merit and concluded that the actual maximum mean milk yield comes at 8.12 years. The following is quoted from their paper: "In these Jersey cows the absolute amount of milk produced per unit of time increases with the age of the cow until a maximum is reached, but the amount of increase diminishes each year with advancing age until the absolute maximum production is reached."

Later, Gowen (3) studied 1741 complete eight-months lactation periods of Jersey cows from records in a single herd. He writes as follows: "The mean milk production for the eight-months period for those cows which are between 2 and 3 years of age, is 4032.9 pounds. From this point the milk production rises rapidly at first then more slowly to a maximum at about 7 years. From this maximum the decline in milk production is less rapid toward the higher ages." Here is a variation of about one year in the age of maximum production of cows of the same breed.

In 1921, Hooper (4) reported the results of a study of 1497 Jersey records from the 1916 Volume of the Register of Merit, and in the following year McCandlish (5) added further knowledge on the subject of the effect of age on production. The latter used a total of 5772 Jersey records from the Register of Merit to July, 1916. These were grouped by ages according to the year in which the test began. The author concludes that the maximum milk and butterfat yield is reached at about eight years of age, and that the fat percentage of Jerseys rises from one to four years and then decreases irregularly.

Most of the foregoing work was criticised by Ragsdale, Turner, and Brody (6) because of the limited data employed. They consolidated 195,678 records from the Jersey Register of Merit, Shorthorn Record of Merit, and Ayrshire, Guernsey, and Holstein Advanced Registers into a composite curve showing the effect of age on production. In the individual breed studies they used 13,723 Jersey records, and the 565 animals from eight to nine years of age show the highest average butterfat production.

The purpose of the present study was to determine the effect of age alone on milk and butterfat production of Register of Merit Jersey cows. Although the material used has been criticised because of the selective influence which Register of Merit requirements exert on official records, yet the fact remains that one of the principal uses to which the results are to be applied is the adjustment of Register of Merit records to a standard age; and it would appear that correction factors derived from the same source as that to which they are to be applied are more nearly accurate than those from any other source. Needless to say, age relationship as determined from more than 14,000 Register of Merit records would likely be sufficiently characteristic of the Jersey breed to make the application of these correction factors to any Jersey records reasonably accurate.

As has been shown elsewhere (7), the increase in production from an initial to a reentry record is due partly to increased age and partly to the development which the animal undergoes during the initial test. This development averages 11 per cent

of the reentry record for Register of Merit Jerseys. Because of this fact all reentry records have been omitted from this study of the influence of age on production, and the results are based on an analysis of 14,571 initial Class-A and Class-AA Jersey records contained in the Register of Merit up to and including the 1921 Volume. By discarding the reentry records the distorting influence of the factor of development is eliminated, as it will be shown that certain age groups have decidedly higher proportions of reentry records than others, thereby weighting the influence of development in these groups. Furthermore, the inclusion of reentry records tends to introduce the additional influence of selection exercised by the breeders in retesting more of their better cows (7). This would again raise the average of the age groups having the greater proportion of reentry records.

In the case of Gowen's (3) study of 1741 private records, it goes without saying that those in charge of the herd where the records were made used the heifer records as a basis for culling the nonproducers. If they failed to do so, then what was the purpose of the testing? If no low producers were eliminated, it must be assumed that there was an unusual increase in the number of females; or that cows were sold in their early maturity regardless of producing ability; or that the death rate equaled the birth rate, which would indicate a condition of herd health such as to make the records of doubtful value for statistical analysis.

Our data confirm a well established popular belief that the maximum producing ability of dairy cows extends over a period of several years after they attain maturity. In most previous studies it has been shown that the age of maximum production is reached at some fixed time, such as 8 years, 1 month, 13 days. That any certain breed or any individual cow would reach her maximum producing capacity on such a narrowly prescribed time limit does not seem logical. Athletes, as rule, during a period of their lives are capable of performing at a fairly uniform rate; and the same is true of race horses. Utmost physical ability in most lines of endeavor as well as mental capacity continue unabated over a period of time.

Butterfat

525

500

475

450

425

400

375

350

325
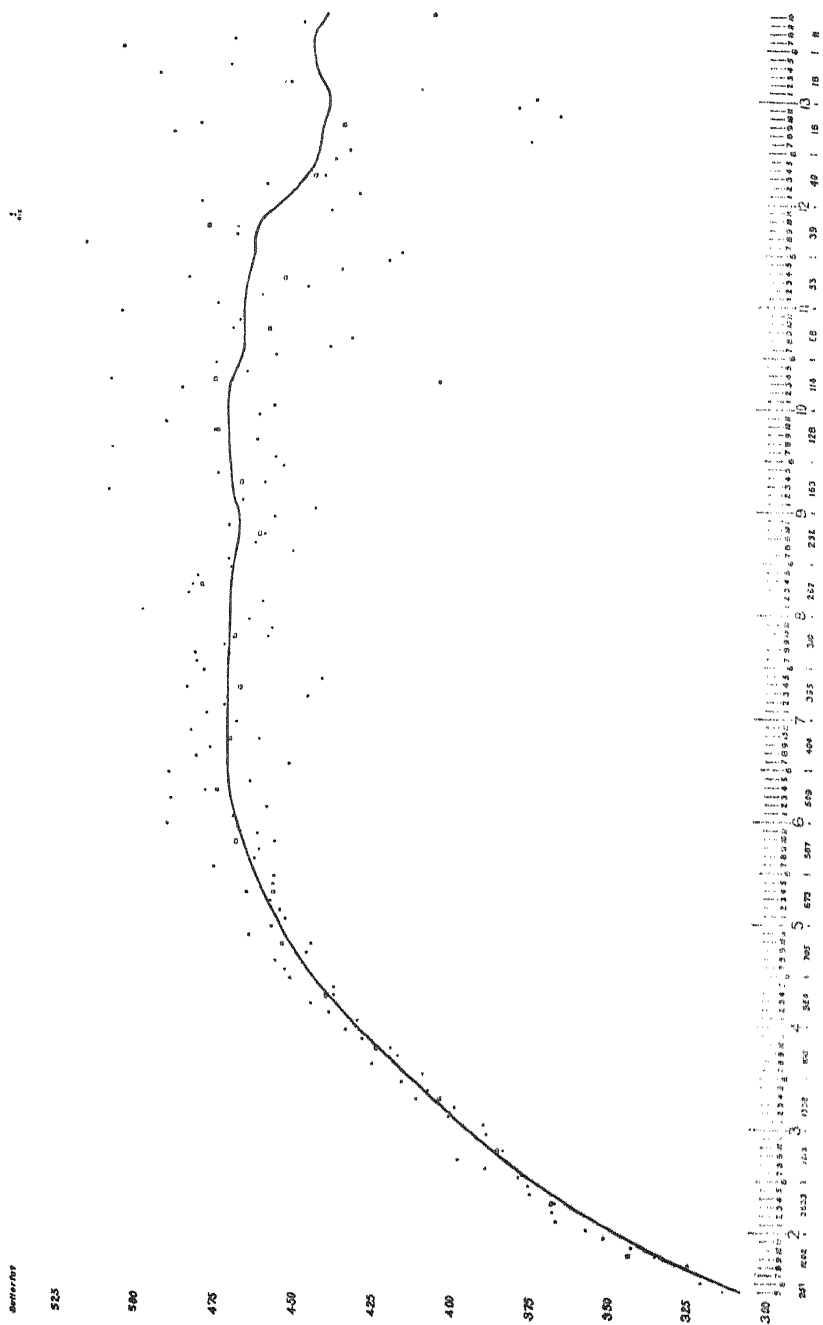
FIG. 1. INFLUENCE OF AGE ON BUTTERFAT PRODUCTION
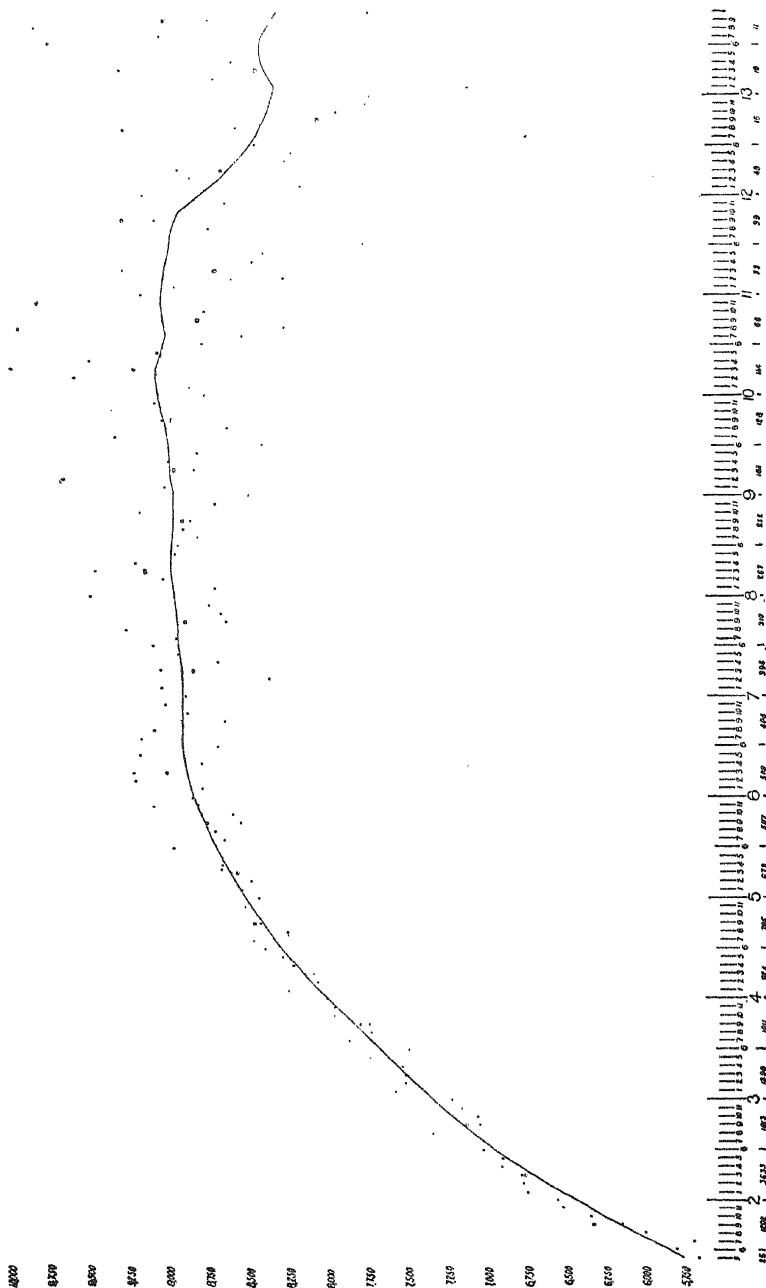
Fig. 2. Influence of Age on Milk Production

473
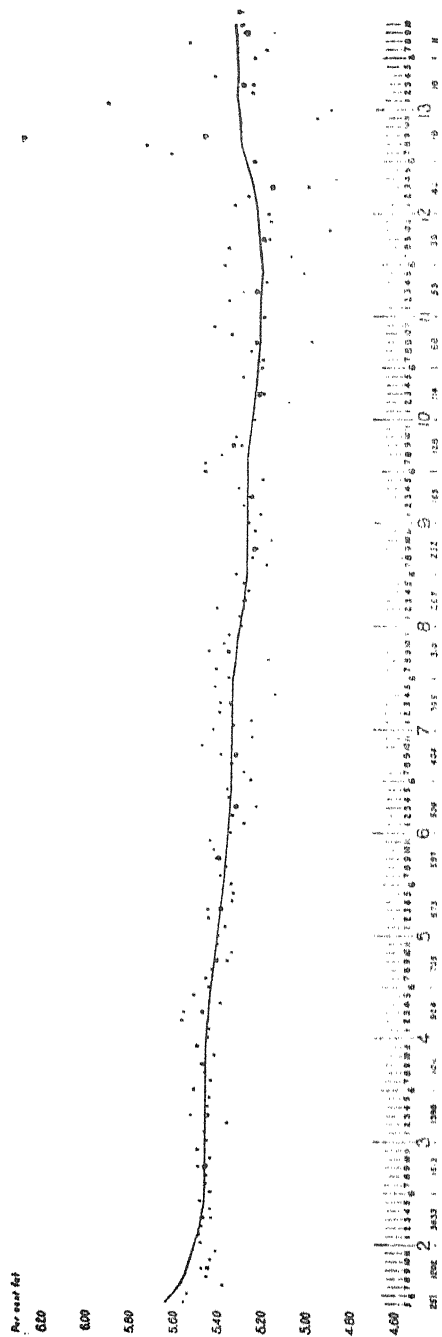
FIG. 3. INFLUENCE OF AGE ON PER CENT OF FAT

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If due allowance is made for environmental influences, which limit the value of all official records, the minor fluctuations in records of cows tested more than once during mature life can be fully accounted for.

Figures 1, 2, and 3 show the curves of butterfat production, milk production, and percentage of fat, as evolved from the Register of Merit data already described. The curves of milk and butterfat production show that there is a period of maximum production from the age of 6 to $10\frac{1}{2}$ years.

The trend of both the milk and butterfat production curves shows a sharp change at about the age of 6 years. The curve of milk production continues to rise slowly until about $10\frac{1}{2}$ years of age, when the trend changes. However, the total increase in milk during this period of $4\frac{1}{2}$ years is about 200 pounds, which may be attributed largely to the influence of three small groups (ages 10-2, 10-3, and 10-4) numbering only 45 animals, and including five records above 13,000 pounds of milk. A further contributing factor to this increase at the ages of 9 and 10 years is that the animals officially tested at advanced ages are more closely selected, and only animals of unusual ability are tested.

The influence of the declining percentage of fat is sufficient to offset the slight rise in milk after 6 years and to change the butter fat production curve to a horizontal, which remains practically so until the age of $10\frac{1}{2}$ years.

The percentage of fat shows an almost uniform decline until after 11 years, and the succeeding portion of the curve is based on groups with limited numbers. This corresponds to Gowen's (8) observations on percentage of Jerseys.

The type of the curves of milk and butterfat production confirms the belief that the period of maximum production of Jersey cows continues from 6 to $10\frac{1}{2}$ years of age.

Unquestionably the shape of these curves would have been changed by the inclusion of reentry records, which were omitted for the reasons previously stated. Table 1 shows the number and average initial records used as compared with the number and average records which would have been added to each group if the reentry records had been included. The younger classes

receive little or no influence from the relatively higher reentry records. The table shows that every class from $4\frac{1}{2}$ up to $8\frac{1}{2}$ years would have had over 50 per cent additional records averaging from 14.3 per cent to 20.5 per cent higher than the initial records.

To facilitate the work of adjusting Register of Merit records to a standard age basis, table 2, containing factors for correcting

TABLE 1

Initial and reentry records of Register of Merit Cows

AGE	INITIAL RECORDS USED		REENTRY RECORDS OMITTED		RATIO OF REENTRY TO INITIAL RECORDS	DIFFERENCE BETWEEN INITIAL AND REENTRY
years	*number*	*pounds*	*number*	*pounds*	*per cent*	*pounds*
Under 2	1237	343	0			
2 to $2\frac{1}{2}$	3633	368	0			
$2\frac{1}{2}$ to 3	1313	386	51	412	3.9	26
3 to $3\frac{1}{2}$	1298	404	367	468	28.3	64
$3\frac{1}{2}$ to 4	1011	423	374	490	37.0	67
4 to $4\frac{1}{2}$	924	440	422	505	45.7	65
$4\frac{1}{2}$ to 5	705	454	370	532	52.5	78
5 to $5\frac{1}{2}$	673	457	372	525	55.3	68
$5\frac{1}{2}$ to 6	587	469	326	543	55.5	74
6 to $6\frac{1}{2}$	509	475	268	543	52.7	68
$6\frac{1}{2}$ to 7	404	471	237	543	58.7	72
7 to $7\frac{1}{2}$	395	468	215	564	54.4	96
$7\frac{1}{4}$ to 8	310	470	185	553	59.7	83
8 to $8\frac{1}{2}$	267	480	140	565	52.4	85
$8\frac{1}{2}$ to 9	232	462	93	573	40.1	111
9 to $9\frac{1}{2}$	163	468	77	559	47.2	91
$9\frac{1}{2}$ to 10	128	475	63	549	49.2	74
10 to $10\frac{1}{2}$	114	476	40	530	35.1	54
$10\frac{1}{2}$ to 11	68	459	29	574	42.6	115

milk and butterfat records, has been prepared from our data. The relatively slight variation in percentage of fat due to age does not warrant the application of correction factors, unless they be used in a gross way; furthermore, there are factors for adjusting both milk and butterfat records. The correction tables are prepared for ages in years and months for reasons which will be demonstrated. Criticism that this involves too much detail can readily be answered by the results shown in

applying various correction factors.   Unless records are standardized with care and precision, results will be badly distorted. For example, suppose two heifers complete records of 500 pounds of butterfat.   Heifer A began her test at 2 years and 10 days, while heifer B started at 2 years, 5 months, and 10 days of age.

TABLE 2

Factors for correcting Register of Merit Jersey records to mature age basis

Procedure: Multiply the actual record by the factor shown opposite the age at which the record was made in order to obtain the age-corrected record.

AGE		MILK FACTOR	BUTTERFAT FACTOR	AGE		MILK FACTOR	BUTTERFAT FACTOR	AGE		MILK FACTOR	BUTTERFAT FACTOR
years	*months*			*years*	*months*			*years*	*months*		
1	5	1.55	1.51	3	1	1.21	1.18	4	9	1.07	1.05
1	6	1.53	1.50	3	2	1.20	1.17	4	10	1.06	1.04
1	7	1.50	1.47	3	3	1.19	1.16	4	11	1.06	1.04
1	8	1.48	1.44	3	4	1.18	1.15	5	0	1.05	1.03
1	9	1.45	1.41	3	5	1.18	1.15	5	1	1.05	1.03
1	10	1.43	1.39	3	6	1.17	1.14	5	2	1.05	1.03
1	11	1.41	1.37	3	7	1.16	1.13	5	3	1.04	1.03
2	0	1.39	1.35	3	8	1.15	1.12	5	4	1.04	1.02
2	1	1.37	1.33	3	9	1.14	1.11	5	5	1.03	1.02
2	2	1.35	1.31	3	10	1.14	1.11	5	6	1.03	1.02
2	3	1.33	1.30	3	11	1.13	1.10	5	7	1.03	1.02
2	4	1.32	1.28	4	0	1.12	1.09	5	8	1.02	1.01
2	5	1.30	1.27	4	1	1.11	1.09	5	9	1.02	1.01
2	6	1.29	1.25	4	2	1.11	1.08	5	10	1.02	1.01
2	7	1.28	1.24	4	3	1.10	1.07	5	11	1.02	1.01
									years		
2	8	1.26	1.23	4	4	1.09	1.07	6 to 10½		1.00	1.00
2	9	1.25	1.22	4	5	1.09	1.06	10½ to 12		1.01	1.02
2	10	1.24	1.21	4	6	1.08	1.06	12 to 14		1.05	1.06
2	11	1.23	1.20	4	7	1.08	1.05	14 and over		1.14	1.12
3	0	1.22	1.19	4	8	1.07	1.05				

In a rough classification both would be called junior two-year-olds.   Applying correction factors in Table 2 gives the following results: Heifer A, 500 × 1.35 = 675 pounds, mature equivalent; heifer B, 500 × 1.27 = 635 pounds, mature equivalent.   The difference is 40 pounds of butterfat.

If the conversion factor of Turner, Ragsdale, and Brody (9)

were applied, both heifers A and B would have mature equivalent records of 725 pounds of fat, or 50 pounds more than A and 90 pounds more than B, as determined above.

It might be of interest to apply the correction factors to a number of Register of Merit records. As a comparative group of Register of Merit cows, the various class leaders have as a common distinction the fact that each is supreme in her own age division. In table 3 are the actual and age-corrected records of the 365-day class leaders as listed in the Jersey Bulletin of July 29, 1925.

TABLE 3

CLASS	NAME	BUTTER-FAT RECORD	AGE		AGE-COR-RECTED RECORD
		pounds	years	months	pounds
Yearling....	St. Mawes Lad's Lady	829	1	11	1,136
Jr. 2........	Raleigh's Torono Meme	902	2	5	1,146
Sr. 2........	Sensation's Mikado's Millie	850	2	8	1,046
Jr. 3........	Poppy's Dortha	994	3	4	1,143
Sr. 3........	St. Mawes Lad's Pride	1,002	3	7	1,132
Jr. 4........	Darling's Jolly Lassie	1,141*	4	0	1,244
Sr. 4........	Vive La France	1,031	4	7	1,083
Mature.....	Groff's Constance	1,130	5	3	1,164

* Highest record for the Jersey breed.

The most butterfat which any Jersey cow has ever produced during a year while an official test is 1141 pounds. This figure indicates the utmost ability of the best cow among some 22,000 cows which have been admitted to the Register of Merit. Of all the animals over five years of age, which are classed as mature, the best producer has a record of 1130 pounds of butterfat. The various class leaders are the best producers in their respective age divisions, and when the age-correction factor is applied to each record it is interesting to observe how closely the adjusted records approximate the highest of the breed. The record of Darling's Jolly Lassie, when corrected for age, exceeds anything which has yet been achieved by a Jersey cow; but it is not inconceivable that this is an exceptionally precocious and

early maturing animal; and unless she demonstrates at a later date an increased ability, her record as a four-year-old marks her full capacity to produce butterfat. Records of all other classes, when adjusted for age, group fairly well around the actual high record for the breed. The average age-corrected record for the eight class leaders is 1137 pounds—only 4 pounds below the actual record of Darling's Jolly Lassie, and 7 pounds in excess of the actual record of Groff's Constance, the highest producer among the cows over five years of age.

By way of comparison with the above, the same records and the adjusted records secured by use of the tables of Turner,

TABLE 4

CLASS	ACTUAL RECORD	AGE		ADJUSTED RECORD—TURNER, RAGSDALE, AND BRODY	ADJUSTED RECORD—DAVIDSON
	pounds	years	months	pounds	pounds
Yearling	829	1	11	1,227	1,227
Jr. 2	902	2	5	1,308	1,218
Sr. 2	850	2	8	1,139	1,105
Jr. 3	994	3	4	1,243	1,203
Sr. 3	1,002	3	7	1,162	1,182
Jr. 4	1,141	4	0	1,278	1,301
Sr. 4	1,031	4	7	1,113	1,134
Mature	1,130	5	3	1,187	1,198
Average				1,207	1,196

Ragsdale, and Brody (9), and one developed by Davidson (10) from data previously cited (2) are listed in table 4.

Six of the adjusted records in each list is in excess of the highest record ever made, 1141 pounds. One corrected record in each list exceeds 1300 pounds of butterfat, and three others in each are above 1200 pounds. Of the thousands of mature cows which have undergone Register of Merit tests, Groff's Constance tops the list with 1130 pounds. If such marked ability existed as is shown by the age-corrected records of young cows in these two lists, similar ability and higher records for the mature class should be found. Unless the records adjusted from earlier ages agree

fairly well with what the cows actually produce when mature, then the correction factors are at fault.

SUMMARY

A table of factors for adjusting Register of Merit Jersey milk and butterfat records to a standard age basis has been evolved from the initial records found in the Register of Merit up to and including the 1921 Volume.

The result of the application of these factors to actual records is compared with results secured by the use of other correction factors.

The production curves of Register of Merit cows indicate that the period of maximum producing ability continues from 6 to $10\frac{1}{2}$ years of age.

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THE EFFECT OF HEATING ON THE HYDROGEN-ION CONCENTRATION AND ON THE TITRATABLE ACIDITY OF MILK*

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INTRODUCTION

Surprisingly little work has been reported on the changes in hydrogen-ion concentration and in titratable acidity which occur when milk is heated. Since the first factor is a major determinant in the coagulation of milk, and since both should give evidence as to the character and extent of chemical changes involving acids, a determination of these values over a considerable range of temperature and time of heating is desirable.

PREVIOUS WORK

Kirsten (1) found a decrease in the titratable acidity of milk after heating for a short time at various temperatures. Centrifuging milk caused a somewhat smaller decrease in titratable acidity. Heating of the centrifuged milk caused a still further drop. He showed that these changes could be correlated with and accounted for by the loss of carbon dioxide.

Orla-Jensen and Plattner (2) found that by heating milk for five minutes at temperatures as high as 110°, a decrease in titratable acidity was obtained; at higher temperatures an increase was found. Heating for an hour at 110° gave an increase. A development of color in the milk accompanied the increase in titratable acidity. They explained the decreases in titratable acidity by loss of carbon dioxide; the increase by development of acid, largely from the casein and to a less degree from lactose.

Van Dam (3) found an increase in hydrogen-ion concentration and a decrease in titratable acidity after heating milk for periods

of not more than an hour. He attempted no explanation of this seeming paradox, but took the hydrogen-ion change to indicate that the calcium caseinate is not hydrolyzed in the heating of milk, and hence the greater stability of heated milks cannot be explained on the basis of such an hydrolysis.

Duncombe (4) reported values for titratable acidity and hydrogen-ion concentration on milks measured *at* different temperatures. He found both values increased by raising the temperature. These measurements are very significant, but, until the temperature range of reliable measurement of hydrogen-ion concentration can be extended much higher than is now possible, the value of such figures is rather limited and attempts at their interpretation unsafe.

Cosmovici (5) heated milk for periods of thirty minutes at temperatures of 56°, 68°, 75° and 100° and states that the hydrogen-ion concentration was increased to an extent dependent on the temperature and time of heating, and that the change was irreversible.

Apparently no one has measured the changes in these values over any extended period of heating. We are making such measurements and report here on two typical experiments, one at the boiling point of milk, the other at 95°.

<div align="center">EXPERIMENTAL</div>

For each of the experiment reported here, 3 liters of fresh, separated herd milk were used. The initial pH in one experiment was 6.55, in the other 6.61. The round-bottom flask, in which the heating was carried out, was provided with a reflux condenser and a thermometer and was immersed to above the level of the surface of the milk in a bath of saturated salt solution in which was suspended a second thermometer. In the 95° experiment, the temperature of the salt bath was raised to about 100° and held there till the milk had reached 90°, after which it was held at 95 to 96°. In the experiment at boiling temperature, the bath was raised to 106 to 107° and maintained there. Samples were removed hourly by means of a syphon, which could be blown clear after each sampling. The samples were cooled immediately

to room temperature by means of ice-water and the necessary determinations made at once.

The hydrogen-ion determinations were made in duplicate in Clark rocking electrode vessels, a saturated calomel half-cell completing the chain. A Type K Leeds and Northrup potentiometer was used for the measurements. A lead accumulator was used as a source of potential for the comparisons, and this potential was kept at a constant known value by frequent balancing against a Weston cell which had been calibrated by the Bureau of Standards. The value of the calomel half-cell was checked each day before using by means of an M/20 solution of potassium hydrogen phthalate. The milk samples were cooled to the temperature of the measuring system before determining their hydrogen-ion concentration. Results were not recorded until the duplicates in the two cells checked to 0.01 pH unit.

A preliminary experiment showed that it was not practicable to titrate the heated milk with phenolphthalein as indicator, because of the peculiar brownish color which masked the end-point to increasing degrees as the heating progressed. Therefore, we decided to titrate electrometrically to an end-point at pH 8.0, which is the end-point most people obtain approximately by using phenolphthalein in milk.

A Hildebrand electrode immersed in the milk in a jelly glass was used for the titrations. The saturated potassium chloride bridge was a piece of glass tubing drawn to an upward-pointing fine tip at the end immersed in the milk. The bridge had in it a stopcock that was kept closed during titrations. The other half of the chain was a saturated calomel half-cell. The same potentiometer, accumulator, and standard cell were used as in the hydrogen-ion determinations.

To a 50 cc. sample of the milk cooled to the temperature of the measuring system was added N/10 sodium hydroxide till near the end-point. The electrode and bridge were then immersed and the potentiometer adjusted to the null point. Two minutes were sufficient for the attainment of equilibrium when the electrode was previously saturated with hydrogen. Standard alkali was then added slowly till equilibrium was obtained at the poten-

tiometer reading corresponding to pH 8.0. No difficulty was found in duplicating results. The end-points were checked in a few cases by making hydrogen-ion determinations on the titrated samples with the Clark electrodes.

DISCUSSION

Figures 1 and 2 show the results obtained. In plotting the curves, the time when the milk reached the temperature at which it was subsequently maintained was taken as zero time. The values at the left of the zero line are the values obtained on the unheated milk. Titratable acidity has been plotted as cubic centimeters of N/10 sodium hydroxide solution necessary to bring 100 cc. of milk to pH 8.0. Hydrogen-ion concentration has been plotted as actual normality of hydrogen ions times 10^6, to avoid the common difficulty of grasping the magnitudes of the differences between the logarithmic values of pH.

The milk at the higher temperature (fig. 1) was visibly coagulating after thirteen hours heating. The shape of the hydrogen-ion concentration curve seems to indicate that the beginning of coagulation was about two hours earlier. The milk held at 95° had not coagulated when the heating was discontinued. This lower temperature curve is included mainly to show the differences in the rates of change of hydrogen-ion concentration and titratable acidity at different temperatures.

The explanation of previous workers that evolution of carbon dioxide is the cause of the initial drop in titratable acidity of heated milk is without doubt the true one. That this effect is superimposed upon and completely masks for a brief time the production of acid from some constituent of the milk is a point that has been reported only by Orla-Jensen and Plattner (2). Their statement that this acid comes mostly from casein decomposition and to only a slight extent from lactose is supported by titratable acidity values on unheated and heated lactose solutions and on heated casein solutions with and without lactose present. They do not give titratable acidities on their casein solutions before heating; they do not state the technique employed in their casein titrations; nor is it clear to what extent their

casein was identical with the casein of fresh milk. We propose to attack this problem from a different angle and hope to corrobo-
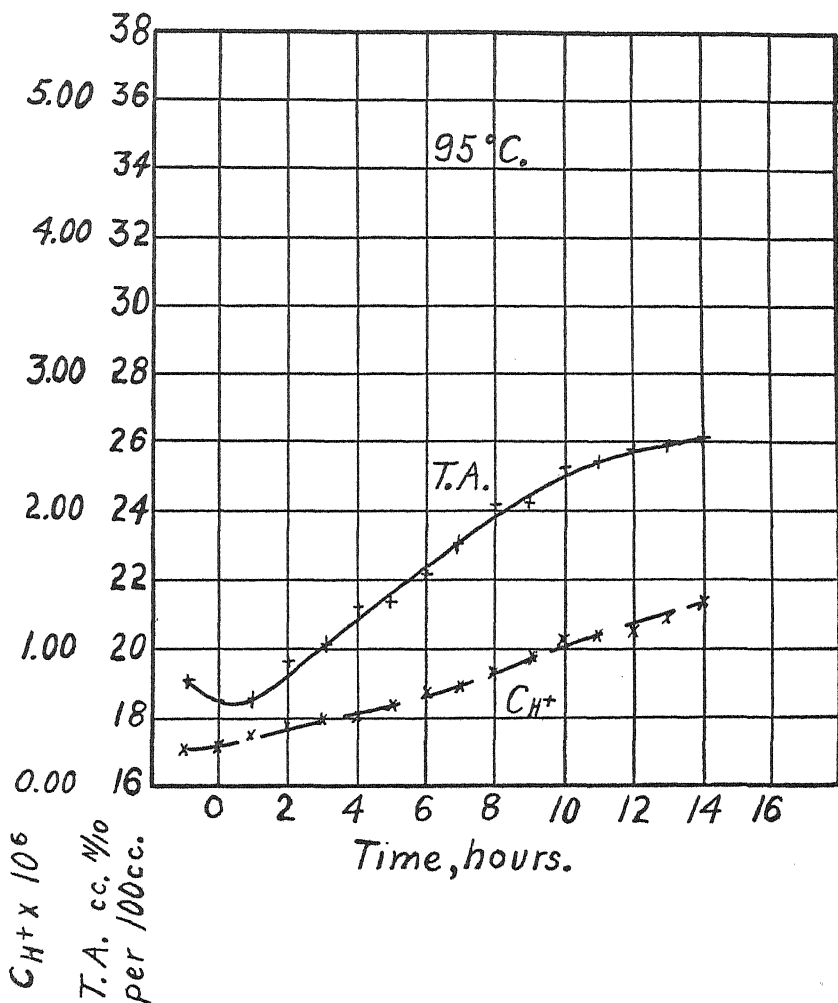
FIG. 1. EFFECT OF HEATING AT 101.5°C. ON THE HYDROGEN-ION CONCENTRATION AND ON THE TITRABLE ACIDITY OF MILK AT 95°

rate or to disprove their conclusion on a somewhat more substantial basis. An explanation of the reversing curvature of the titratable acidity curve during the first twelve hours heating may

be easier when the source and identity of the acids formed is more firmly established.   It should be noted that the rate of production
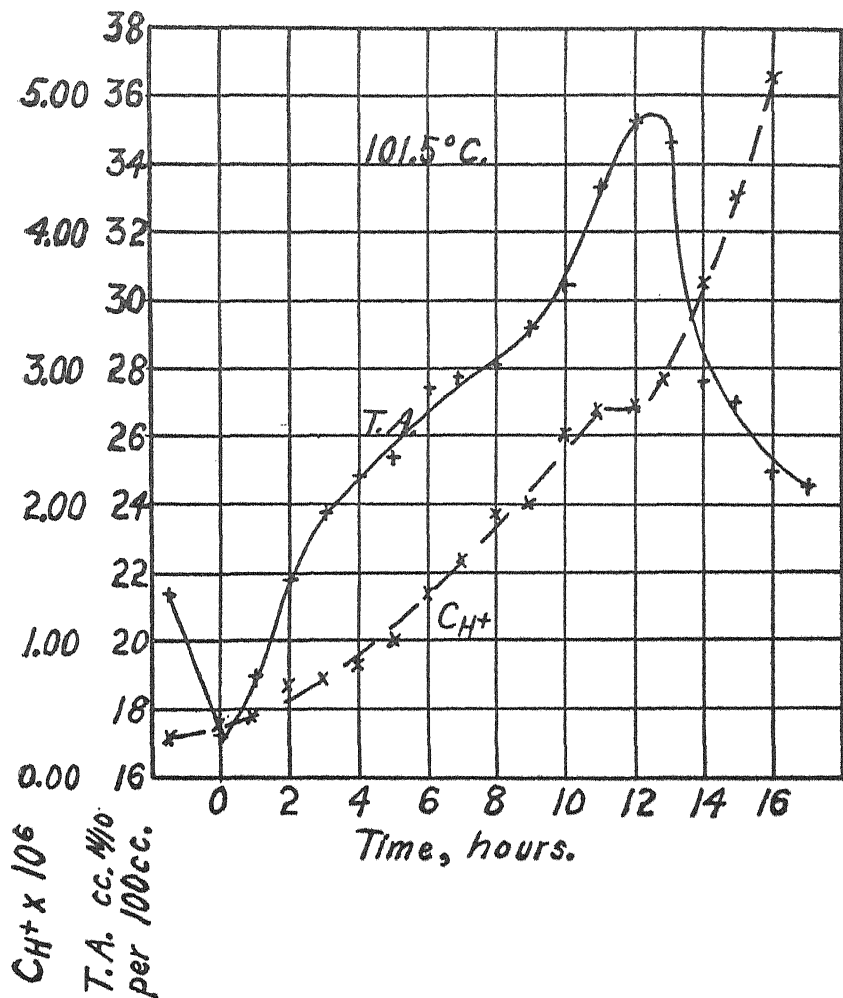
FIG. 2.   EFFECT OF HEATING AT 95°C. ON THE HYDROGEN-ION CONCENTRATION AND ON THE TITRATABLE ACIDITY OF BOILED MILK

of acid at 95° is much less than at the boiling temperature, but the curvature for the first fourteen hours at 95° is comparable with that for the first seven hours at 101.5°.

The decrease of the titratable acidity after twelve hours boiling represents the change in acidity in the fluid portion from which increasing amounts of coagulum have separated. Titration of a sample of the curdy portion, which had separated roughly from the supernatant fluid at the end of the run, gave a titratable acidity value of 50.9 cc. N/10 acid per 100 cc., showing that the acid concentrates in the curd, probably by adsorption.

The increase in the hydrogen-ion concentration caused by heating skim milk is fairly regular, as might be expected from the assumption that it is the result of the interaction of the buffer system of the milk with acids that are being produced at a fairly constant rate. During the early stages of coagulation there is evidently a considerable increase in the buffering power for a short time. The hydrogen-ion concentration then reëstablishes itself on a sharper slope, and at the end of sixteen hours has reached a point which it would have reached had it continued smoothly at the curvature it was following during the first eleven hours of heating. The decrease of hydrogen-ion concentration which takes place when heated milk is allowed to stand at low temperatures, and which has been previously noted, was observed. Samples which stood in the ice-box for about twenty-four hours showed a decrease numerically equivalent to the increase caused by two hours heating. This would indicate a shift of buffer equilibrium with change of temperature. The magnitude of this reversion is so small that it is of only theoretical interest.

CONCLUSIONS

The heating of skim milk at temperatures near the boiling point causes first a drop and then a rise in the titratable acidity of the milk. The hydrogen-ion concentration increases continuously. The initial drop in titratable acidity has been shown by previous investigators to be due to loss of carbon dioxide from the milk; the increases in titratable acidity and in hydrogen-ion concentration are due to the formation of acids from certain constituents of the milk. During coagulation, the rate of change of hydrogen-ion concentration is considerably lessened due to

buffer readjustments not yet explained in detail. At the same time, there is an uneven distribution of the free acid between the whey and the curd, due probably to adsorption of the acid by the curd.

The amount of acid production is dependent on both time and temperature of heating. We do not agree entirely with previous workers as to the sources of the acid, and shall present in a future paper experimental data bearing on the nature of the chemical reactions involved.

REFERENCES

(1) KIRSTEN: Z. Nahr. Genussm., v, 97 (1902).
(2) ORLA-JENSEN AND PLATTNER: Landw. Jahrb. Schweiz., xix, 235 (1905).
(3) VAN DAM: Milchwirtschaft. Zentr., v, 154 (1909).
(4) DUNCOMBE, J.: Dairy Sci., vii, 245 (1924).
(5) COSMOVICI: Compt. rend. soc., biol., xcii, 73 (1925).

AN APPRECIATION

A distinct tribute was paid Dr. C. H. Eckles, Head of the Dairy Department, University of Minnesota, during Farmers' Week when the Eckles Club, composed of his former students, presented an oil painting of their "Chief." This portrait was unveiled and hung in the new dairy building of the University on January 22.

The presentation was made by C. M. Long of the Blue Valley Creamery Institute, who fully expressed the sentiments of the club when he said: "The members of the Eckles Club feel that they owe a debt of gratitude to their Chief bigger than they will ever be able to pay. Desiring to express in some small way this debt, they have had this portrait painted."

Dr. Eckles' former students may be found wherever dairy cows are milked and dairy products are manufactured. They are engaged in all branches and in all phases of the dairy industry. In his teaching and investigational work, first at the University of Missouri and later at the University of Minnesota, he has had greater influence on the practice and profession of dairying than any other living man today.

The sole purpose of the Eckles Club is to meet once a year at the National Dairy Show for a friendly get-together and to receive counsel and advice from the "Chief." There are now about 115 members and the membership is increasing at the rate of about five each year.

Dean W. C. Coffey of the College of Agriculture, University of Minnesota, in accepting this portrait for the University made the following statement:

I doubt if any other man in agricultural education now or in the past has trained as many men who have risen to responsible positions as has Professor Eckles. His standing in the educational field as it related to dairying is unequaled and his influence in that field now is

profound. He is quiet, unassuming, always modest, always cheerful, always fair. His power lies in keeping his well trained mind at work on objectives he clearly understands, and in giving all he has to his position and to the young men who work and study under him. With him the course of friendship runs deep; it is never ephemeral with him, and he never uses superficial methods to get it.

In these young men who constitute the Eckles Club, he has accumulated great riches which will continue to grow as he unselfishly shares them with the world. How paltry is mere money beside them! It is with this feeling,—a feeling, by the way, to which every member of the faculty on this campus would instantly subscribe—that I accept this portrait for the Department of Agriculture, University of Minnesota.

ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The twenty-first annual convention of the American Dairy Science Association will be held at Detroit, October 8 and 9.

The Book-Cadillac Hotel will be association headquarters and there all meetings will be held. Single rooms are $4; doubles, $6. Other good hotels are the Wolverine, Tuller and Fort Shelby.

Here are the high points in the meetings:

October 8: Morning; general meeting, addressed by Pres. Butterfield of the Michigan State College.

October 8: Afternoon; sectional meetings.

October 8: Evening; banquet, address by Secretary of Agriculture Jardine, and Judging Contest awards announced.

October 9: Morning; general session.

THIS IS GOING TO BE A BIG SHOW. Besides the usual attractions of the National Dairy Exposition, the greatest trade exhibit in the history of the country will be staged. Several convention records will be broken. Let's all go and make it a record breaker for the A. D. S. A.

Faithfully,

G. C. WHITE,
Secretary-Treasurer.

PROGRAM

GENERAL SESSION

BOOK-CADILLAC HOTEL, DETROIT, MICH.

Friday, October 8, 1926, 9:00 a.m.

Call to Order by President..................O. E. Reed, East Lansing, Mich.
Reading of Minutes by Secretary..................G. C. White, Storrs, Conn.
Secretary and Treasurer's Report..................G. C. White, Storrs, Conn.

Appointment of Nominating Committees.
Divisional Reports: Eastern Division, Southern Division, Western Division.
Reports of Standing Committees.
Address......................Dr. Kenyon L. Butterfield, East Lansing, Mich.
Discussion: The Development of the Work of the American Dairy Science
    Association.

GENERAL SESSION

BOOK-CADILLAC HOTEL

Saturday, October 9, 1926, 9:00 a.m.

Call to Order by President...................O. E. Reed, East Lansing, Mich.
Report of Sections:
    Section I.................................C. C. Hayden, Wooster, Ohio
    Section II................................A. C. Baer, Stillwater, Okla.
    Section III.............................A. J. Cramer, Madison, Wisc.
    Section IV...............................C. E. Wylie, Knoxville, Tenn.
Committee Reports.

BANQUET

NEWCOMB ENDICOTT TEA ROOM

Friday, October 8, 1926, 6:30 p.m.

Toastmaster................................H. F. Judkins, Springfield, Mass.
Address......Hon. W. M. Jardine, Secretary of Agriculture, Washington, D. C.
Announcement of Awards and Presentation of Prizes in the InterCollegiate
    Judging Contests.

(Banquet tickets $1.50 per plate)

PROGRAM

SECTION I. PRODUCTION

Friday, October 8, 1926, 2:00 p.m.

C. C. Hayden, Ohio, *Chairman*
O. G. Schaefer, Minnesota, *Secretary*

What we know about controlling abortion disease......Dean G. C. White, Storrs,
    Conn.
What experiments to date show about minerals for dairy cows
    Dr. E. B. Forbes, Director, Institute of Animal Nutrition, State College, Pa.
Report of committee on future coöperative work in mineral investigations
    Dr. E. B. Meigs, Bureau of Dairy Industry, Washington, D. C.

Business.
Reports of Judging Committee, at banquet.

SECTION II. MANUFACTURING

Friday, October 8, 1926, 2:00 p.m.

A. C. Baer, Oklahoma, *Chairman*
C. D. Dahle, Pennsylvania, *Secretary*

Call to Order by Chairman
Reading of Minutes by Secretary

Reports of Standing Committees:

National Contest for Judging Dairy Products.............R. B. Stoltz
Score Cards and Legal Standards.....................J. H. Frandsen
Chemical Methods of Testing Dairy Products.............A. C. Dahlberg
Methods of Determining Milk Solids-not-fat.................H. C. Troy
Testing Ice Cream for Total Solids......................R. C. Fisher
Official Methods of Testing Milk and Cream................F. W. Bouska
Bacteriological Methods....................................R. S. Breed
(a) Sub-Committee on Ice Cream...........................A. C. Fay
College Creameries...................................C. L. Roadhouse
Economic Phases of the Dairy Industry...................Roy C. Potts
The effect of processing on the dispersion of fat in an ice cream mix
                              Wm. H. E. Reid, Columbia, Mo.
Discussion: Relation of sugar to overrun and quality
                              P. L. Lucas, East Lansing, Mich.
Discussion: Some new developments in ice cream making
                              H. H. Sommer, Madison, Wis.
Body of Butter................................E. S. Guthrie, Ithaca, N. Y.
Discussion—(a) Body as it affects moisture holding quality....F. W. Bouska
            (b) Effect of working on the body, etc.........O. F. Hunziker
Uniformity of methods of manufacture of cottage cheese
                    Carl E. Lee, Gridley Dairy Company, Milwaukee, Wisc.
Discussion: Preservation of cottage and cream cheeses in vacuo
                              J. M. Sherman, Ithaca, N. Y.
Discussion: Cream Cheeses....................A. C. Dahlberg, Geneva, N. Y.

SECTION III. ADVANCED REGISTRY
Friday, October 8, 1926, 2:00 p.m.
C. Elmer Wylie, Tennessee, *Chairman*
W. E. Petersen, Minnesota, *Secretary*

Minutes of last meeting.
Report of Breed's Relation Committee..M. H. Campbell, Chairman, Illinois.
Report of Investigation Committee.......Roy Harris, Chairman, Wisconsin.
History and Trend of Official Testing...........Dr. C. H. Eckles, Minnesota.
The Ayrshire Herd Test Plan
                    C. T. Conklin, Secretary, Ayrshire, Breeders Association
Supervising the Herd Test Plan...........Prof. P. S. Williams, Pennsylvania
Remarks by Breed Association Representatives:
    American Jersey Cattle Club
    American Guernsey Cattle Club
    Ayrshire Breeders Association
    Brown Swiss Cattle Breeders Association
    Dutch Belted Cattle Association of America
    Holstein-Friesian Association of America
    Shorthorn Breeders Association
Reports of Special Committees.
Election of Officers.

SECTION IV. EXTENSION
(Program has not been received.—*Editor*)

AMERICAN DAIRY SCIENCE ASSOCIATION
MEMBERSHIP FOR 1926

The following is a list of the membership of the American Dairy Science Association for 1926. Any member whose name does not appear in this list should communicate at the earliest convenience with Secretary G. C. White, Storrs, Conn.—*Editor*.

ADAMS, C. F......................5014 E. Seventh Street, Kansas City, Mo.

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ALLEN, C. L.....................Cornell University, Ithaca, N. Y.

ALTWEGG, C. A..................Gluckskille Milchgesellschaft, Neustadt in Holstein, Germany

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The Association now has 326 members. While this is the largest membership in its history, the list is far to small to be commensurate with the economic importance of the industry, and the vastness of its problems. The Agronomists, the Horticulturists, and many other similar groups, far outstrip us in membership enrollment. We have arrived at a point where personal work is necessary for further expansion. In a few institutions almost every eligible person is enrolled, while in others we scarcely have representation. If each member will interview those with whom he has influence, the enrollment can easily be doubled. This will greatly increase the financial strength of the organization. Will you not consider yourself a committee of one to bring at least one new member?

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Faithfully,

G. C. WHITE,
Secretary-Treasurer.

</div>

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BOOK REVIEW

CONDENSED MILK AND MILK POWDER—a new and completely revised edition by Professor Otto F. Hunziker—is just off the press. This work is thorough and comprehensive as is everything from the pen of Professor Hunziker.

The author treats exhaustively all phases of sweetened condensed milk—unsweetened condensed milk—milk powder—and dry buttermilk. He describes methods of manufacture—causes of defects—keeping quality—and best methods of marketing of all these products.

The popularity of this book may be judged by the fact that this is the fourth edition, the three previous editions having long since been exhausted. The book is the most important contribution on the condensed milk and milk powder industry. It should be in the library of the teacher, the student or factory man interested in any phase of the condensed milk and milk powder industry. Copies can be secured direct from the author at LaGrange, Illinois.

STUDIES ON YEASTS IN DAIRY PRODUCTS

I. RELATIONSHIPS OF YEASTS TO DAIRY PRODUCTS[*]

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The budding organisms found in milk and its various derivatives are becoming of increasing concern to investigators interested in dairy products. While the known importance of these organisms in causing certain undesirable changes, such as the formation of gas in cream and in sweetened condensed milk, accounts in part for this, their possible relationship to abnormal conditions in cream not yet definitely ascribed to any particular organism, to the deterioration of butter, and to various other undesirable changes makes their study very necessary.

The ability of yeasts to grow under conditions that must be considered unfavorable for the growth of many bacteria is one of the reasons for their importance in dairy products. Certain types of molds can also withstand severe conditions such as high acid, dearth of water, etc., in the materials on which they are growing but their air requirements limit their growth in a way that the development of certain yeasts is not limited. Moreover the growth of molds, because of their development only in rather close contact with air, is usually readily evident on observation.

A number of yeasts grow in the presence of large amounts of acid. If the temperature is suitable certain yeasts can grow rapidly throughout a can of old fermented cream in which the growth of many types of bacteria has been stopped by the acid concentration, and, as a result, abnormal flavors and odors due to yeasts are extremely common in the cream coming to the creameries to which cream is shipped for considerable distances. The acid tolerance of yeasts also enables them to grow on acid curd cheeses, such as cottage and neufchatel, on certain cheeses in which ripening occurs, such as camembert, in starters, etc.

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On some of these materials, especially acid curd cheeses and starters, definite colonies or areas of growth may be formed when the products are held at temperatures that are at all favorable for growth. The lactose-fermenting yeasts are of particular importance in acid cream because they produce gas under conditions that rather effectively limit the growth of other gas-producing organisms; they cause serious losses, not only from the influence they have on the flavor and odor of the material but also because the gas commonly forces cream from the cans. In certain types of cheese, such as Swiss, the lactose-fermenting organisms also sometimes cause serious losses by producing a swelling of the product.

Some yeasts not only tolerate considerable acid but are actually favored by it and grow better in sour milk than they do in sweet milk. With certain forms the production of objectionable odors occurs much quicker and is more pronounced in milk which contains some lactic acid, either added as such or produced by S. lactis, than in milk of the same lot without acid.

Other factors that are ordinarily considered to limit the growth of microörganisms seem to have less effect on yeasts than on many other forms. Certain yeasts grow in high concentrations of NaCl and while some types of bacteria do the same thing, these latter organisms apparently are not of importance in dairy products. The production of gas in sweetened condensed milk in which the sugar is often supposed to prevent the growth of organisms is commonly due to yeasts. Although certain cocci can grow in sweetened condensed milk, their growth is comparatively slow and is of much less importance because of their usual failure to produce significant changes.

Unfavorable conditions do not seem to result in the destruction of yeasts in the way they do many bacteria. For example in butter held for considerable periods many of the bacteria may have died out while the number of yeasts may remain comparatively high and be equal to or larger than the original count. Certain cocci of course also have the ability to resist the conditions found in butter. In laboratory cultures, yeasts are commonly found in a living condition when the medium is dried until there is only a small amount along the wall of the tube.

The large size of yeast cells in comparison to that of bacterial cells suggests that a smaller number of cells would be present in material that had been fermented by yeasts than in material fermented by bacteria. This has been found to be the case and accordingly the isolation of yeasts from material showing an abnormal condition due to these organisms requires much less dilution than if bacteria are being isolated. When bacteria are present, as they are very likely to be in dairy products, and the plates are poured so as to give the usually desired distribution of colonies with these organisms, the yeasts may be largely missed. The common procedure is to restrain the bacteria by some such method as the addition of acid and then plate heavily enough to give a satisfactory number of yeast colonies per plate. The acidity which will restrain bacteria and allow yeasts to grow is however not definite. One cubic centimeter of a 1 per cent tartaric acid solution per plate (of 8 to 10 cc. of media) is commonly employed with quite satisfactory results but it does not by any means entirely limit the growth of bacteria. As amounts of acid above this are employed there is a gradual falling off in the number of yeast colonies developing and it seems that any reasonable amount of acid—that is one which will allow a good development of yeasts—will also allow the development of certain types of bacteria. Most of the bacteria encountered in yeast isolations however are types whose colonies can readily be distinguished from yeast colonies since these in general admit of the individual cells being seen along the edge of the colony with the low power of the microscope. Occasionally colonies of large cocci may be mistaken for yeast colonies but stained preparations quickly show the error by the lack of budding cells and by the smaller size.

The yeasts common in milk and its derivatives cannot be looked upon as types peculiar to these products. Although yeasts have been found in milk drawn aseptically, in general these organisms get into milk after it leaves the udder so that there must be sources of them about the stables, milk houses, etc. The yeasts common in dairy products are accordingly to be expected in other materials which have not been studied in

any detail from the standpoint of their flora, because they are products whose value is not readily influenced by microörganisms. Since such materials are important sources of microörganisms, however, their flora must eventually be studied before a clear understanding of the yeasts of dairy products can be secured.

A number of the yeasts common in dairy products do not produce visible changes when grown in milk, and this is particularly surprising because certain of them are sometimes present in very large numbers so that growth must have taken place. Whether these organisms are of no significance from the standpoint of dairying or whether their products are of importance in the deterioration of such materials as butter, etc. can only be determined after the organisms are better understood.

Among the yeasts common in dairy products there are certain groups that seem to be clearly defined as natural groups. The lactose-fermenters for example stand out quite definitely from the other yeasts; these organisms are clearly separated from the other forms because of their gas production in milk and moreover it seems that this separation is particularly significant because when gas production occurs it is very definite, and slow or questionable fermentations are not likely to occur. Accompanying the gas production there is an alcoholic odor and, in the case of litmus milk, a reddening of litmus, both of which are uncommon changes for yeasts to produce in milk. These organisms also are almost always present in milk or cream produced under the usual conditions although during the colder weather, when their growth is slow if it occurs at all, considerable amounts of the milk or cream must often be employed for the isolation.

The yeasts producing a pink color, while frequently found in materials other than dairy products, are so common in these that they are entitled to considerable attention. These organisms apparently are never present in large numbers in ordinary milk or cream but frequently form colonies on such materials as old cream, cottage cheese, starters that are being held, etc. The pink color of these forms seems always to be definite enough so that colonies can readily be detected, whether they are on dairy products or on some plating medium. This type is further characterized by the pink color occurring as a ring or sediment in

milk tubes and by the slow changes, other than the colored areas, produced in milk.

The yeasts causing a rapid digestion of milk constitute another type that is clearly defined because outside of this type the rapid digestion of milk by yeasts is not common. Moreover the colonies produced by these organisms are quite characteristic and in general the organism can readily be picked out by the colony alone.

The yeasts that are responsible for the blowing of sweetened condensed milk constitute a definite group that is perhaps most clearly characterized by the ability to grow in strong sucrose solutions. With these types should be included other organisms that can grow in concentrated sugar solutions but which have not been isolated from spoiled sweetened condensed milk. It would be expected that these organisms might be of importance in materials other than sweetened condensed milk.

While many yeasts have been isolated from dairy products and described, these descriptions are not usually based on a study of a number of cultures from different sources that are to be considered as a single species, and accordingly the descriptions commonly do not take into account the variations that occur in the particular type. Moreover, many of the descriptions are so incomplete, particularly with reference to the action of the organism on milk, that they are of little value from the standpoint of identification. As a result, it is extremely difficult to isolate yeasts from dairy products and identify them as having been previously described and named. The confusion is being increased by certain investigators designating a yeast in published results by one of its characteristics such as "the rapidly liquefying yeast," "small white yeast," etc.

The dairy section of the Iowa Agricultural Experiment Station has been interested in the yeasts of dairy products for a number of years and has published data dealing with certain of these organisms; the forms that have been considered are, however, unrelated, and have been investigated only because of some special significance attached to them. Other types of yeasts have been studied in more or less detail and it is planned to publish data and descriptions with reference to certain of these from time to time.

RESULTS OF PRELIMINARY EXPERIMENTS UPON THE EFFECT OF SEPARATING, OR CLARIFYING, AND PASTEURIZING OF A MILK UPON THE KEEPING QUALITY OF ITS MILK POWDER.*

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In a previous report of work from these laboratories (1)[1] it has been shown that the treatment such as evaporation and homogenization, received by a milk previous to manufacture into powder produces marked changes in keeping quality. The results reported also seemed to show that increased fat content lowered the keeping quality.

The observation has been made that powdered cream possesses a keeping quality superior to that of a whole-milk powder. This appears contrary to the results reported in the publication cited.

In seeking an explanation for this, it seemed probable that the only factor wherein cream differs from whole milk is that it has gone through a centrifuging process, or has been clarified, whereas whole milks do not always get this treatment prior to the drying process. There was a probability also that the removal of slime, containing leucocytes and various enzymes, might have some effect upon the susceptibility of the fat to oxidation.

EXPERIMENTAL

A large quantity of milk with 3.8 per cent fat content was divided into 3 parts. Part 1 was given only pasteurization treatment before powdering. Parts 2 and 3 were separated and all the slime from both was returned to sample 2. No. 3 therefore was the clarified sample. Parts 2 and 3 were treated similarly to Part 1 in regard to pasteurization and were then powdered. In

order to make the results comparable to the usual industrial practice, the ordinary pasteurization temperature and time were used (63°C. for thirty minutes). This procedure resulted in greater variations in the values for the different samples than might have been the case had higher temperatures been used. The samples were placed in an atmosphere of oxygen at 70°C., and the time necessary to cause the fat to absorb O_2 is its induc-
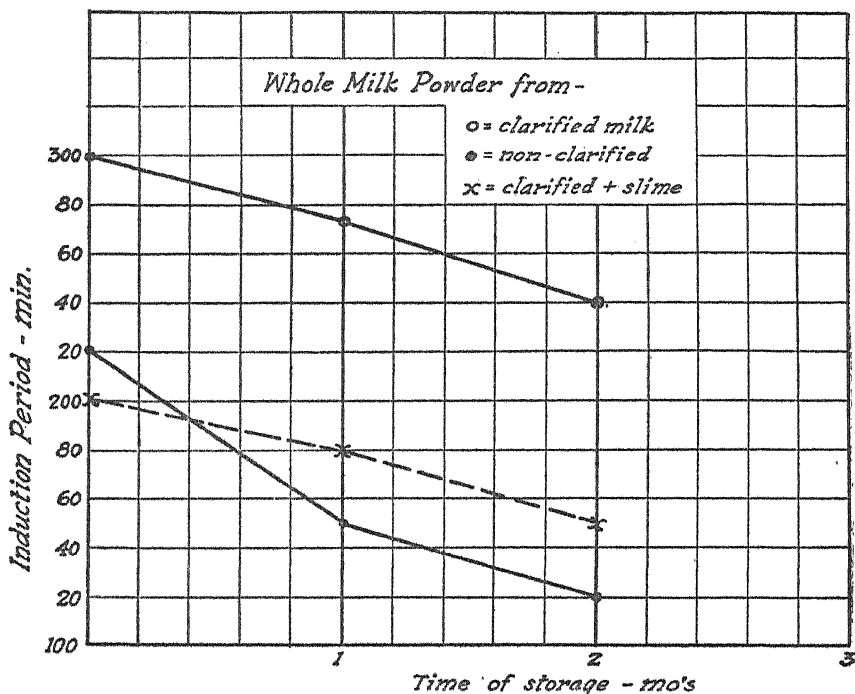
FIG. 1. SHOWING THE EFFECT OF CLARIFICATION OF A MILK UPON THE RESISTANCE OF THE FAT IN ITS POWDER TO OXIDATION

tion period for that temperature. It affords a relative method (2) for following the changes in the resistance of a fat to oxidation. Figure 1 gives the results obtained with these samples upon storage.

The check sample (nonclarified) as well as the sample which contained the separator slime deteriorated rapidly and showed

off flavor after twenty-one days' storage at room temperature. The clarified sample not only showed a good resistance to oxidation (long induction period) as soon as it had been manufactured, but at the end of two months' storage it was sweet and in good condition.

The claim is not made here that this proportionately increased keeping quality can be imparted to all milks by separating or clarifying the milk to be used; yet there is no doubt that the keep-
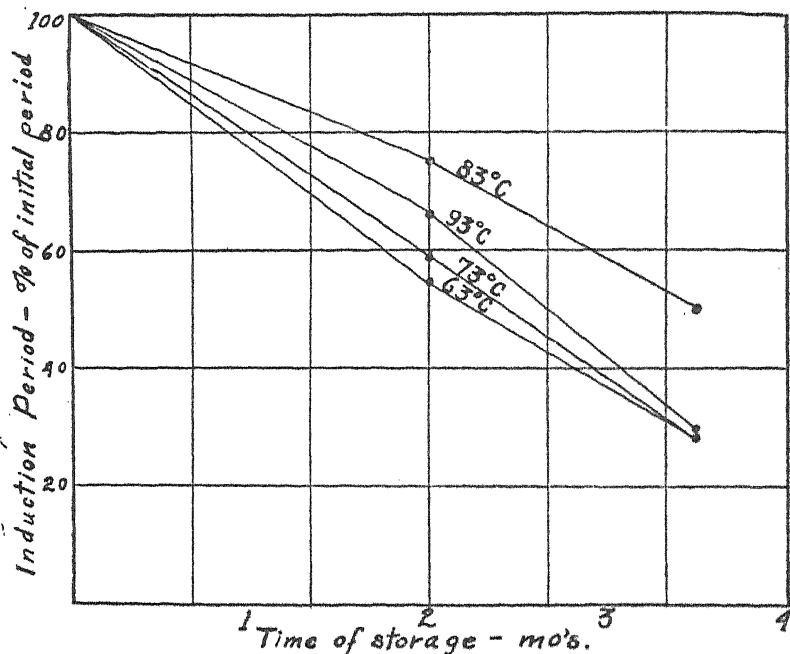
FIG. 2. SHOWING THE EFFECT OF VARIOUS TEMPERATURES OF PASTEURIZATION OF A MILK UPON THE RESISTANCE OF THE FAT IN ITS POWDER TO OXIDATION

ing quality can be materially improved by applying this principle to the treatment of the milk. It is evident that when higher pasteurization temperatures are used, the proportionate effect of centrifuging will become less and less, since higher temperatures tend to lessen enzymic activity.

In order to ascertain the relative quantitative effect of various

temperatures of pasteurization, four samples of whole dry milk were prepared from evaporated herd milk of 3.8 per cent fat content. The milk of sample No. 1 was pasteurized at 63°C., of sample No. 2 at 73°C., of sample No. 3 at 83°C., and of sample No. 4 at 93°C., for thirty minutes each. The samples were sealed in baby cans and stored at room temperatures, and the induction period was measured from time to time. The results are indicated in figure 2.

DISCUSSION

The experiments with powder from clarified milk seem to explain satisfactorily the increased stability of the fat in dried cream over that of the fat in whole-milk powders manufactured under the usual methods. The results perhaps also explain the non-deterioration of the small amounts of fat in skim-milk powder.

Results of experiments by Supplee (3) which seemed to show that the powders of higher fat content possessed the best keeping quality, can probably be explained by the fact that in order to obtain the powders of higher fat contents, cream was added to the milk. Thus in increasing the fat content, a fat of superior keeping quality was added.

The experiments upon clarification also put additional emphasis upon storage of milk prior to its use, as well as separation of the cream as soon as possible after the milk is drawn. It has been noted repeatedly in these laboratories that butteroil isolated from milk held for one day is more susceptible to oxidation than is the butteroil isolated from the milk held less than 12 hours. These observations, as well as the experiments reported, indicate that for the manufacture of products wherein the keeping quality of the fat is the main criterion to be considered, the time of holding should be as short as possible.

Increased temperatures of pasteurization favor increased keeping quality. A temperature of 95°C. for thirty minutes, however, seems to render the product less stable. Though this temperature is not so severe as those used in a previous work (4) to ascertain the effect of heat upon the susceptibility of fats and oils, it may well be the case that even at this temperature the detrimental effect of heat upon stability is shown.

Though preliminary in nature the results discussed, as well as numerous observations upon the keeping qualities of powders from various milks, indicate that the keeping quality of whole-milk powders can be materially improved by clarification of the milk, preferably as soon as possible after it is drawn. Higher pasteurization temperatures than are ordinarily used (63°C. for 30 minutes) also seem desirable, while too high temperatures of pasteurization (95°C.) seem to render the fat more susceptible to oxidation.

Further experiments upon an industrial scale will prove the relative value of the factors discussed to the industry.

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LACTOSE SOLUBILITY AND LACTOSE CRYSTAL FORMATION

I. LACTOSE SOLUBILITY[*]

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FUNDAMENTAL FACTORS IN LACTOSE SOLUBILITY

The experiments reported and results quoted in this paper on lactose solubility refer particularly to lactose solubility in the presence of sucrose and milk colloids as compared with lactose solubility in water.

In the determination of lactose solubility the chief limiting feature is the very slow rate of solution and rate of crystallization of this sugar.

This is due, as explained by Hudson (1), to the fact that lactose can exist in solution in two forms, namely as hydrated lactose and as anhydrous lactose. The stable and generally available form is the lactose hydrate. When milk sugar is placed in water in excess of the amount that is capable of going into solution, a certain portion of the sugar dissolves immediately. This represents the hydrate form of sugar in solution and is termed the initial solubility of lactose. The initial solubility is very low, amounting, according to Hudson (2) to about 8.9 parts of lactose to 100 parts of water at 25°C.

As soon as all the hydrate sugar has gone in solution that is capable of dissolving immediately, the hydrate sugar in solution begins to change to the anhydrous form, and simultaneously more of the hydrate goes into solution. This change from hydrate to anhydride and the further dissolving of hydrate continues until a point of equilibrium of the two forms in solution is reached. This equilibrium is expressed as follows:

$$C_{12}H_{24}O_{12} \rightleftharpoons C_{12}H_{22}O_{11} + H_2O$$
Hydrate   Beta anhydride
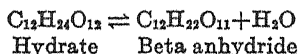
and is attained when for every part of hydrate in solution, there exists about $1\frac{1}{2}$ parts of anhydride in solution. When this equilibrium is established there is no further solution of milk sugar. The milk sugar solution has reached its point of saturation. This represents the final solubility. This change from hydrate to anhydride is very slow and because of this the equilibrium and final solubility are reached only after long periods of dissolving. The same principle underlies the crystallization of lactose from supersaturated solution, the rate of crystallization depending on the change of anhydride into hydrate lactose. This change also is only gradual, hence the complete crystallization of the lactose present in excess of saturation, reducing the state of supersaturation to a state of saturation, is very slow.

GENERAL PROCEDURE OF INVESTIGATION

To test out the effect of milk colloids and sucrose on the solubility of lactose various solutions were prepared in which lactose was dissolved in water, skim milk, whole milk, and in sucrose solutions of the above, respectively.

Materials used. The materials used in this investigation consisted of Merck's C. P. lactose, ranging in moisture content from 4.89 to 5.09 per cent and "Domino" brand sucrose with a moisture content of 1.5 to 2.0 per cent. Difco lactose was also used in one set of experiments, but as no material difference was noted it was decided to continue with only one brand of each sugar. All water solutions were prepared with distilled water. The whole milk used had a total solids content of 11.8 to 12.5 per cent and a fat content of 3.0 to 3.5 per cent, the total solids in the skim milk ranged from 8.69 to 10.26 per cent and the fat was 0.08 per cent or below.

In all the solutions the lactose solubility was based on the water content of the mixture rather than on actual percentage, due allowance being made for sucrose and milk solids where such were present.

To prevent the solutions from spoiling 0.5 cc. of 40 per cent formalin was added to each bottle after having determined that

the presence of the formalin in no way affected the solubility of the lactose.

Lactose solubility as determined by direct observation of the saturation point

This method of determining the solubility of lactose consisted of noting the maximum amount of lactose which will dissolve in, or crystallize out from, a prearranged series of solutions of graduated concentrations.

The method used consisted of shaking a series of 8 ounce bottles sealed with well-paraffined cork stoppers previously boiled in paraffine to prevent evaporation, in a thermostatically controlled shaking chamber whereby the temperature of the bottles could be maintained constant at the temperatures used in the experiment, i.e., at 50°F. and 65°F., respectively. The lactose solubility end point was determined by noting the presence or absence of undissolved lactose or lactose crystals in the bottom of the bottles, avoiding the necessity of chemical analysis for lactose.

After a few preliminary determinations with the various solutions to be used, to roughly determine the approximate working range, it was possible to limit the number of bottles necessary to between 8 and 12 for each type of solution.

Determination A. Exactly 100 grams of water were weighed into each of 12 bottles and then after cooling to the working temperature definitely weighed amounts of lactose were added, ranging from 17.5 to 21 grams in portions differing by 0.25 gram.

These bottles were then continually shaken at constant temperature for from two weeks to one month or more. They were examined at definite intervals during this time to note the progress of solution. Complete solution required 170 hours. The experiment was, however, always continued for at least two weeks to insure complete equilibrium and final solubility.

Determination B. This was conducted in exactly the same way and with the same amount of water and lactose in each of twelve

bottles as in A but in addition 16.6 grams of sucrose were also added to each bottle. This amount of sucrose is equivalent to exactly 14 per cent, which is comparable to the sucrose content of ice cream mix and also to the approximate sucrose contained in the uncondensed milk used in manufacture of sweetened condensed milk. The sucrose in each case was completely dissolved prior to adding the lactose to avoid seeding effect.

Determination C. Still other trials of the same type as above were conducted but using as the solvent instead of water, enough whole milk and skim milk, respectively, to have present in each bottle exactly 100 grams of water. Thus for whole milk approximately 114 grams and for skim milk 110 grams were necessary.

In order to make due allowance for the lactose originally present in the milk, which was found by gravimetric analysis to be from 5.4 to 6.0 grams to each 100 grams of water in the milk, the amount of lactose added was decreased accordingly. The lactose thus added ranged from 11.5 to 15.5 grams. The calculations of the final solubility of lactose were made on the basis of the sum of the amount of lactose originally present in the milk and the amount of lactose added extraneously. This then gave results comparable to lactose solubility in pure water solutions.

Determination D. Another set of similar trials was conducted, using the same amount of whole or skim milk respectively as in C, and adding to this 18.4 grams of sucrose completely dissolved prior to the addition of the lactose. This then constituted a 14 per cent sucrose solution in milk.

Determination E. In all of the preceding trials the lactose solubility was approached from undersaturation, or from below, that is, the dry lactose was added to the cold liquid and allowed to dissolve, thus arriving at the saturation point by beginning with less than saturated solutions. In this trial the lactose solubility was determined from above in an endeavor to ascertain if the same point of solubility could be reached as with determinations from below. This was done by heating the liquid to 140°F. and completely dissolving the sucrose and lactose, then cooling to and shaking at 65°F. till equilibrium was reached. It required 5 weeks to reach a point where no more additional bottles

TABLE 1

Solubility of lactose* in water, milk and 14 per cent sucrose solutions

Results of experiment 1, trials A, B, C, D, and E

KIND OF SOLUTION	TEMPER-ATURE	GRAMS LACTOSE PER 100 GRAMS WATER	PER CENT LACTOSE ANHY-DRIDE IN WATER	PER CENT LACTOSE ANHY-DRIDE BY ANALYSIS
	°F.			
Lactose in water............................	50	14.97	13.0	13.02
Lactose in 14 per cent sucrose solution......	50	13.93	12.4	12.63
Lactose in water............................	65	18.34	15.5	15.56
Lactose in water............................	65	18.34	15.5	15.57
Lactose in water............................	65	18.34	15.5	15.66
†Lactose in water crystallized from solution.	65	18.34	15.5	
Lactose in whole milk.......................	65	18.34	15.5	15.8
Lactose in whole milk.......................	65	18.34	15.5	
Lactose in whole milk.......................	65	18.34	15.5	
Lactose in skim milk.......................	65	18.34	15.5	16.1
Lactose in 14 per cent sucrose solution in water.................................	65	17.6	14.95	14.89
Lactose in 14 per cent sucrose solution in water.................................	65	17.6	14.95	14.94
†Lactose in 14 per cent sucrose crystallized from solution...........................	65	18.05	15.26	
Lactose in 14 per cent whole milk with 14 per cent sucrose............................	65	17.4	14.84	
Lactose in 14 per cent whole milk with 14 per cent sucrose............................	65	17.3	14.70	15.52
Lactose in skim milk with 14 per cent sucrose.	65	17.3	14.70	15.7

* The results in above table were obtained as hydrated lactose but to facilitate comparisons they were converted into the anhydride form by multiplying by 0.95 and are expressed as such.

† Solubility determined from "above," i. e., saturation point approached from supersaturated solution as explained in trial E. All other results in this table were obtained by determining solubility from "below," i. e., approaching saturation point by allowing the lactose to dissolve to saturation.

showed lactose deposits. Twenty-four bottles were used for this test and the lactose solubility was determined in both the presence and the absence of sucrose.

The results of Determinations A, B, C, D, and E of experiment 1 are summarized in table 1.

Discussion of results in table 1. The solubility determinations in water were made chiefly for the purpose of standardizing the method of procedure, so as to make all results comparable to Hudson's determination of lactose solubility in water, whose results are shown in table 2.

From the logarithmic graph of Hudson's solubility figures[1] at different temperatures we find that the solubility of lactose at 50°F. is 13.06 per cent or 15.02 grams to 100 grams of water and at 65°F. 15.54 per cent or 18.4 grams to 100 parts of water.

TABLE 2

Final solubility of lactose by Hudson (2)

| TEMPERATURE | FINAL SOLUBILITY | | TEMPERATURE | FINAL SOLUBILITY | |
	Lactose per cent	Parts lactose to 100 parts of water		Lactose per cent	Parts lactose to 100 parts of water
°F.			°F.		
32	10.6	11.9	120	29.8	42.4
59	14.5	16.9	147	39.7	65.8
77	17.8	21.6	165	46.3	86.2
102	24.0	31.5	192	58.2	139.2

Our results for lactose in water as given in table 1 show very close agreement with those of Hudson. At 50°F. the solubility was 13.0 per cent and at 65°F. it was 15.5 per cent. This close agreement indicates that the method used herein is correct and should give equally reliable results of lactose solubility in solutions other than water and which results may be accepted as comparable with Hudson's lactose solubility in water.

In the last column of table 1 are given additional solubility results obtained on some of the bottles containing excess lactose. In the case of water these results were obtained by total solids

[1] By plotting Hudson's figures upon a graph it will be observed that the type of curve obtained in such as to make accurate interpolation difficult. But by converting Hudson's figures into logarithms, the curve obtained becomes almost a straight line from which solubility figures for intervening temperatures may be readily derived.

determinations. These results show very close agreement with the solubility figures given in the second and third columns and thereby offer additional proof of the accuracy of this method of solubility determination. In the case of milk the chemical analyses were made by use of the gravimetric Fehling's method.

These last results on milk and sucrose milk, however, were made under conditions which may have impaired their accuracy somewhat, as some of the fat churned during the shaking and there may have been some tendency for slight inversion of the sucrose.

The results in table 1 clearly show that the lactose solubility in milk and skim milk per 100 parts of water in the milk is the same as in pure water solution. Similar results concerning the relation of colloids to lactose solubility are reported by Lucas and Spitzer (3). The sucrose in 14 per cent solution appears to have a slight solubility-diminishing effect. The per cent lactose dissolved in a 14 per cent sucrose solution was about 0.6 lower than in pure water.

EXPERIMENT 2

Lactose solubility by determination of total solids

This method of determining the solubility of lactose consisted of analyzing for total solids filtered solutions from mixtures containing lactose in excess of saturation.

After preliminary experimentation with various solutions of known lactose and lactose-sucrose concentrations it was found that the following method of drying gave reliable total solids results.

Total solids determination. Either 2 or 5 gram samples of the well mixed, filtered solutions were weighed out by the use of Mojonnier curved pipettes. Lactose solutions containing no sucrose were dried on the plain dish, since no difference was observed by drying on asbestos. Sucrose solutions and sucrose-lactose solutions were all dried on a layer of previously well dried asbestos fibre. Mojonnier solids dishes were used in all cases. The dish was first heated on the outside hot plate (180°C.) till apparently dry, then held for forty minutes in the 100°C. vacuum

oven and then for ten minute intervals till constant in weight. No further loss in weight was observed by subsequently heating at 135°C. unless when carried to the point of excessive browning.

Form of lactose in total solids determinations. Since lactose may exist in both the hydrate and the anhydrous form, there was some doubt as to which of these forms was being obtained. In order to definitely determine this, another series of preliminary determinations was made in which exactly 5 grams of Mercks lactose hydrate were dissolved in 45 grams of water; and 5 grams of sucrose were dissolved in 45 grams of water and a third solution

TABLE 3

Determination of form of lactose from solutions containing lactose, and lactose and sucrose

SAMPLE NUMBER	KIND OF SOLUTION	AMOUNT OF SUGAR ADDED	WEIGHT OF SUGAR ACTUALLY FOUND	CALCULATED WEIGHT OF ANHYDRIDE LACTOSE	DIFFERENCE
	1 gram of lactose hydrate in anhydrous form should weigh			0.9491	
1	10 per cent lactose solution	1.000	0.9437	0.9491	0.0044
2	10 per cent sucrose solution	1.000	0.9910		
3	10 per cent lactose and 10 per cent sucrose solution	2.000	1.9453		
	Lactose in solution containing 10 per cent lactose and 10 per cent sucrose	1.000	0.9543	0.9491	0.0052

was made containing 5 grams of lactose and 5 grams of sucrose in 40 grams of water. Total solids determinations were made on 10 grams of each of the above three solutions. The results of this preliminary trial are shown in table 3.

Discussion of results in table 3. The figures in table 3 show amounts of lactose approximately 5 per cent less than the amount added. This indicates that the total solids determinations obtained by the method used for solubility determinations yield the lactose in the anhydride form. The slight variations shown may well be considered within the experimental error.

It appears, therefore, correct to assume that the product

obtained in the following total solids determinations is the lactose anhydride which, as mentioned earlier, differs from the lactose hydrate form which is stable at ordinary temperatures by being 5 per cent lighter.

This fact was also mentioned by Schmöger (4) who showed that the evaporation of a lactose solution at 100°C. produced anhydrous lactose and that the dry lactose hydrate does not lose water at 100°C.

Hudson (5) also stated that the substance obtained in total solids determinations was a mixture of the alpha and beta anhydrides having the formula $C_{12}H_{22}O_{11}$. And Rice and Miscall (6) have recently demonstrated that lactose appears in the total solids residue from aqueous as well as condensed milk solutions in the anhydrous form.

Since sucrose contains a small amount of moisture, as shown under "Materials Used" earlier in this paper, due allowance was made in preparing the mixture of lactose-sucrose solutions for all lactose solubility determinations.

Total solids determinations for lactose solubility from lactose solutions in water and in 14 per cent sucrose solutions at 50°F. and 65°F. Aqueous mixtures containing lactose in slight excess of saturation and similar mixtures containing 14 per cent sucrose in addition to the lactose were continually shaken at a constant temperature of 50°F. and 65°F., respectively, for two weeks or longer. They were then filtered in an incubator maintained at the same temperature at which the mixtures had been shaken and total solids determinations were at once made on the clear filtrate. In solutions where both lactose and sucrose were present the solubility of lactose in water was obtained from the total solids figure by subtracting the known amount of sucrose previously added. The results of these determinations are assembled in table 4.

Discussion of results in table 4. The lactose solubility results obtained by the total solids determination given in table 4 show very close agreement with the results in table 1 and with Hudson's solubility figures for lactose in water. It appears, therefore, that solubility results obtained from this method of total solids deter-

TABLE 4

Solubility of lactose by total solids determinations of the filtered solutions from mixtures of lactose in water and lactose in 14 per cent sucrose solutions containing slight excess of lactose

Expressed as lactose anhydride $C_{12}H_{22}O_{11}$

SAMPLE NUMBER	KIND OF SOLUTION	TEMPERATURE	PER CENT LACTOSE ANHYDRIDE IN WATER PART OF SOLUTION	GRAMS OF LACTOSE IN 100 GRAMS WATER
		°F.		
1	100 grams of water + 16.0 grams of lactose hydrate	50	12.92 13.00	14.84 14.95
2	100 grams of water + 16.25 grams of lactose hydrate	50	13.19 12.96	15.19 14.89
3	100 grams of water + 16.5 grams of lactose hydrate	50	12.92 12.98	14.84 14.92
4	100 grams of water + 16.75 grams of lactose hydrate	50	13.03 13.05	14.98 15.00
5	100 grams of water + 17.00 grams of lactose hydrate	50	13.02 13.05	14.97 15.01
6	100 grams of water + 16.6 grams of sucrose + 15.5 grams of lactose	50	12.55 12.55	14.35 14.35
7	100 grams of water + 16.6 grams of sucrose + 15.75 grams of lactose	50	12.72 12.74	14.57 14.60
8	100 grams of water + 16.6 grams of sucrose + 16.0 grams of lactose	50	12.84 12.66	14.73 14.50
9	100 grams of water + 16.6 grams of sucrose + 16.5 grams of lactose	50	12.54 12.59 12.58	14.34 14.40 14.38
10	100 grams of water + 21.0 grams of lactose	65	15.53 15.50	18.40 18.43
11	100 grams of water + 20 grams of lactose	65	15.75 15.76	18.69 18.70
12	100 grams of water + 20.5 grams of lactose	65	15.59 15.54	18.46 18.41

TABLE 4 – *Concluded*

SAMPLE NUMBER	KIND OF SOLUTION	TEMPER-ATURE	PER CENT LACTOSE ANHYDRIDE IN WATER PART OF SOLUTION	GRAMS OF LACTOSE IN 100 GRAMS WATER
		°F.		
13	100 grams of water + 21.0 grams of lactose	65	15.55	18.42
			15.55	18.42
14	100 grams of water + 20.0 grams of lactose	65	15.66	18.57
			15.67	18.58
15	100 grams of water + 16.6 grams of sucrose + 19.0 grams of lactose	65	14.73	17.28
			14.68	17.21
16	100 grams of water + 16.6 grams of sucrose + 19.5 grams of lactose	65	14.75	17.30
			14.80	17.37
17	100 grams of water + 16.6 grams of sucrose + 20.0 grams of lactose	65	14.53	17.00
			14.55	17.03
18	100 grams of water + 16.6 grams of sucrose + 19.0 grams of lactose	65	14.86	17.45
			14.80	17.37
19	100 grams of water + 16.6 grams of sucrose + 16.5 grams of lactose	65	14.99	17.63
			14.91	17.52
20	100 grams of water + 16.6 grams of sucrose + 17.0 grams of lactose	65	14.94	17.57
			14.85	17.44
21	100 grams of water + 16.6 grams of sucrose + 18.0 grams of lactose heated to solution and shaken for 5 weeks	65	15.03	17.69
			15.10	17.79

mination are also accurate and should yield reliable solubility figures for lactose in the presence of sucrose.

Table 4 shows that the solubility of lactose is slightly lower in 14 per cent sucrose solution than in water alone.

Lactose solubility by total solids determinations in presence of large excess of lactose. Additional determinations of lactose solubility by the total solids method were made as follows:

Solutions were prepared in 8 and 12 ounce bottles and shaken as previously described, but in this case large excesses of lactose

TABLE 5

Lactose solubility at 65°F.

Solutions prepared with large excess of lactose

SAMPLE NUMBER	KIND OF SOLUTION	TOTAL GRAMS OF LACTOSE ADDED PER 100 GRAMS OF WATER		PER CENT LACTOSE IN WATER	GRAMS LACTOSE IN SOLUTION PER 100 GRAMS WATER
1	Lactose in water	30		15.89 15.86	18.90 18.85
2	Lactose in water	30		16.10 16.13	19.15 19.18
3	Lactose in water	40		16.02 16.00	19.07 19.05
4	Lactose in water	30		16.01 15.99	19.05 19.04
5	Lactose in water	50		16.39 16.40	19.59 19.60
6	Lactose in water	50		16.47 16.42	19.67 19.62
7	*Lactose in water	40		16.52 16.51	19.91 19.90
8	*Lactose in water	50		16.40 16.38	19.62 19.59
9	*Lactose in water	60		16.66 16.65	19.99 19.98
10	Lactose in 14 per cent sucrose solution	30		15.70 15.68	18.63 18.61
11	*Lactose in 14 per cent sucrose solution	40		16.10 16.00	19.17 19.09
12	Lactose in 14 per cent sucrose solution	50		15.61 15.70	18.49 18.63
13	Lactose in 14 per cent sucrose solution	25		15.25 15.25	18.00 18.00

TABLE 5—*Continued*

SAMPLE NUMBER	KIND OF SOLUTION	TOTAL GRAMS OF LACTOSE ADDED PER 100 GRAMS OF WATER	PER CENT LACTOSE IN WATER	GRAMS LACTOSE IN SOLUTION PER 100 GRAMS WATER
14	Lactose in 14 per cent sucrose solution	25	15.72 / 15.70	18.66 / 18.64
15	Lactose in 62 per cent sucrose solution (163 grams sucrose)	35	13.75 / 13.52	15.95 / 15.64
16	*Same as 62 per cent sucrose solution	35	13.59 / 13.52	15.68 / 15.64

* Lactose solubility determined by first completely dissolving all the sugar present at 150 to 170°F. then cooling to the working temperature and allowing crystallization to proceed from the supersaturated solution until no further crystallization took place.

All results not "starred" (*) were obtained by adding the dry lactose to the cold water of the previously prepared sucrose solution and agitating until no further solution of the lactose took place.

were used. The lactose present in excess of saturation ranged from 25 to 200 per cent. After two weeks or more of shaking at constant temperature the solutions were allowed to settle and the clear liquid was poured through a filter, avoiding, as much as possible, evaporation during filtration. The mixtures used consisted of lactose in water and lactose in 14 per cent and 62 per cent sucrose solution, respectively, and total solids determinations were made as shown in table 5.

Discussion of results in table 5. The results in table 5 show slightly higher lactose solubility in solutions containing a large excess of lactose, as evidenced both from below (undersaturation) and from above (crystallization from supersaturation). This fact suggests the possible influence of the law of mass action in slightly advancing the point of equilibrium.

Similarly as in previous determinations with lesser excess of lactose, the presence of sucrose again slightly lowered the solubility of the lactose. This phenomenon becomes even more pronounced in the presence of highly concentrated sucrose solutions, as shown in the case of solutions containing 62 per cent sucrose.

Determination of lactose solubility in milk by total solids method.
An attempt was made to further determine lactose solubility in
milk solutions by the total solids method.

Approximately 114 grams of whole milk and 110 grams of
skim milk respectively, or enough milk to give exactly 100 grams

TABLE 6

Solubility of lactose at 65°F. in milk serum after filtering off the curd

SAMPLE NUMBER	KIND OF SOLUTION	GRAMS OF LACTOSE ADDED PER 100 GRAMS WATER	PERCENT LACTOSE IN WATER	GRAMS LACTOSE TO 100 GRAMS WATER
1	Lactose in whole milk	20	15.90	18.91
2	Lactose in whole milk	25	16.45	19.69
3	Lactose in whole milk	30	16.36	19.56
4	Lactose in whole milk containing 14 per cent sucrose	20	15.69	18.62
5	Lactose in whole milk containing 14 per cent sucrose	25	15.68	18.61
6	Serum solids of whole milk soured as the above	*	5.48	
7	Lactose in skim milk	20	16.2	19.37
8	Lactose in skim milk	20	16.2	19.37
9	Lactose in skim milk	30	15.7	18.58
10	Lactose in skim milk	30	15.9	19.10
11	Lactose in skim milk containing 14 per cent sucrose	20	15.0	17.6
12	Lactose in skim milk containing 14 per cent sucrose	20	15.0	17.6
13	Lactose in skim milk containing 14 per cent sucrose	30	14.89	17.4
14	Lactose in skim milk containing 14 per cent sucrose	30	14.90	17.4
15	Serum solids of skim milk soured as in the above	*	5.87	

* No added lactose.

of water, were shaken with 20 to 30 grams of lactose powder for
two weeks at 65°F.   At the end of this time the casein had curdled
and settled out, as no preservative had been added.   By filtering
off the clear serum and analyzing this for total solids an approxi-
mate estimate may be secured as to the amount of lactose in
solution.   Direct sugar determinations were not deemed advis-

able on these solutions due to the condition of the samples. These results are summarized in table 6.

Discussion of results in table 6. Since the milk serum separated in the manner described contains in addition to the lactose also some serum solids not lactose, amounting to about 1.5 grams per 100 grams of serum, this amount was deducted from the actual total solids figures obtained and the results are thus recorded as lactose solubility in table 6.

These figures show considerably greater variations than those previously obtained. This might be expected on account of the difficulty of preserving the original physical character of the milk during such long agitation periods.

It had been intended to determine lactose solubility by difference in total solids before and after the period of shaking but this was prevented by the churning of the fat and slight change in the casein which made filtration and centrifuging of the lactose from suspension very difficult even where preservatives were present. Representative samples of the milk as such were, therefore, considered to be out of the question and the solids determinations were made on the serum from these milk samples, which really yields the desired results, i.e., the lactose solubility in the water portion of the milk. However, the results obtained and given in table 6 may serve to show that the amount of lactose dissolved in the watery part of the milk (the serum) is practically the same as shown by results previously obtained in milk and in aqueous solutions.

EXPERIMENT 3

Lactose solubility determinations by gravimetric Fehling's method. In solutions containing very high sucrose content, such as solutions with a sucrose content of 62 per cent as is approximately found in sweetened condensed milk, and also supersaturated sucrose solutions used in this experiment, it was deemed somewhat unreliable to determine lactose by the methods previously given in this paper. The content of both sugars was, therefore, determined by the regular gravimetric Fehling's solution method using Munson and Walker's tables.

These solutions were prepared by completely dissolving the two sugars in a water bath at 150° to 170°F. and, after cooling, maintaining a temperature of 65°F. for six weeks with occasional shaking. Total solids determinations were made at intervals on the supersaturated liquid and when no further change could be detected the lactose crystals in suspension were removed by centrifuging until no crystals could be detected under the microscope. The top portion of the liquid was then stirred carefully and siphoned off as completely as possible into a dry bottle. In the case of the aqueous solutions no difficulty was experienced but in the case of the milk solutions it was necessary to repeat the centrifuging as some lactose crystals still appeared in suspension after the first separation.

The solutions prepared were as follows:

1. Sixty-two per cent sucrose-water solution containing 100 grams of water, 163 grams of sucrose and 35 grams of lactose.

2. Sixty-two per cent sucrose-milk solution containing 113.6 grams of whole milk, 163 grams of sucrose and 35 grams of lactose.

3. Supersaturated sucrose-water solution containing 100 grams of water, 230 grams of sucrose and 35 grams of lactose.

4. Supersaturated sucrose-milk solution containing 113.6 grams of whole milk, 230 grams of sucrose and 35 grams of lactose.

After cooling, the water lost by evaporation during the heating was replaced. The water of replacement contained 0.5 cc. of formaldehyde so as to check fermentation.

Preliminary lactose and lactose-sucrose determinations using known solutions of Difco lactose and Pfanstiehl chemically pure sucrose in water and in milk, of about the same sugar ratio in water as those of the experiment proper showed that no factor was necessary in determining sucrose in such solutions even in the presence of lactose, but that in the case of determining lactose in the presence of sucrose the 1 lactose-12 sucrose column of table 7 A. O. A. C. Methods (7) should be used, and that in addition the results must be multiplied by the factor 1.0173 and also by 0.95 to express the results as anhydrous lactose. All results were obtained in triplicate. The results are assembled in table 7.

Discussion of results in table 7. Since the solubility of lactose in 62 per cent sucrose-water solution above given agrees well with that obtained from total solids determinations, see table 5, this serves as an additional check on lactose solubility by the total solids determination.

These results further show that sucrose in high concentrations such as solutions containing about 62 per cent sucrose, does affect the solubility of lactose in water to a considerable extent reducing it approximately 15 per cent. Thus, as shown in table 1, 100 grams of pure water at 65°F. will dissolve approximately 18.4 grams of lactose, while 100 grams of water of a 62 per cent sucrose solution will dissolve only about 15.35 grams lactose.

TABLE 7

Solubility of lactose in 62 per cent and supersaturated sucrose water and whole milk solutions at 65°F.

Determined by Gravimetric Fehling's Method. Expressed as anhydrous lactose ($C_{12}H_{22}O_{11}$).

KIND OF SOLUTION	TOTAL SOLIDS OF WHOLE	PERCENT SUCROSE IN WATER OF SOLUTION	PERCENT LACTOSE IN WATER OF SOLUTION	GRAMS LACTOSE TO 100 GRAMS OF WATER
	per cent			
Water containing 62 per cent sucrose....	63.65	61.40	13.20	15.20
Whole milk containing 62 per cent sucrose	63.93	61.57	13.45	15.50
Supersaturated sucrose-water solution...	68.37	66.67	14.49	16.94
Supersaturated sucrose-milk solution....	69.10	66.91	14.01	16.30

This is significant in connection with sweetened condensed milk, since sweetened condensed milk which contains from 40 to 45 per cent sucrose usually has a sucrose-in-water concentration of from about 60 to 64 per cent. Sweetened condensed milk contains from about 11 to 15 per cent total lactose and from about 24 to 28 per cent water. On the basis of these figures the lactose-water ratio would range from about 28 to 38 per cent while the lactose solubility in this product is only about 13 to 14 per cent or from about 15 to 16 grams to 100 grams of water. It appears evident, therefore, that from about one-half to two-thirds of the lactose contained in sweetened condensed milk is present in excess of saturation, i.e., in crystalline form. The figures in table 7 are

of further interest in that the information they reveal is helpful in the determination of the amount of lactose crystals present in sweetened condensed milk of known composition.

The results in table 7 further show that the milk colloids in sweetened condensed milk have no noticeable effect on lactose solubility in the water portion of the milk. The amount of lactose dissolved in the sucrose milk solution being probably the same as that dissolved in the sucrose water solution, i.e., 13.2 and 13.45 per cent, respectively.

Commercial application of these facts in the manufacture of sweetened condensed milk and the use of such knowledge in controlling the smoothness of the finished product are discussed in detail by Hunziker (8).

Similarly as in the case of 62 per cent sucrose solutions, so did solutions in which the sucrose as well as the lactose was present to the extent of supersaturation, have a solubility-diminishing effect on the lactose. And again, in such solutions in milk the milk colloids appeared not to affect the lactose solubility. However, the lactose solubility results in these sucrose-supersaturated solutions, where both sugars are crystallizing do not show quite as close agreement as those from solutions of lesser sucrose concentration. It is possible that in these concentrated solutions complete crystallization may not have been definitely obtained, although the solutions were allowed to crystallize for two months.

SUMMARY

1. Lactose solubility in water has been rechecked and very close agreement was obtained with the results of Hudson.

2. Lactose solubility determinations in sucrose solutions and in milk with and without sucrose were made. The results of these determinations show that the solubility of lactose in milk was similar to that in aqueous solutions, but that the presence of sucrose decreased the lactose solubility somewhat. This decrease in lactose solubility ranged from about one-twentieth to one-sixth of the lactose solubility found in pure water. It was only slight in dilute sucrose solutions, such as solutions containing 14

per cent sucrose, and it became greater with an increase in the sucrose concentration.

3. Lactose solubility was approached both from below and

TABLE 8

*Lactose solubility averages**

KIND OF SOLUTION	TEM- PERA- TURE	AVERAGE OF BEST RESULTS		AVERAGE OF ALL FIGURES	
		Percent lactose in water part of solution	Grams lactose to 100 grams of water in the solution	Percent lactose in water part of solution	Grams lactose to 100 grams of water in the solution
	°F				
Lactose in water..................	50	13.02	14.97	13.02	14.97
Lactose in water..................	65	15.50	18.34	15.97	19.01
Lactose in whole milk............	65	15.50	18.34	15.87	18.86
Lactose in skim milk.............	65	15.50	18.34	15.90	18.95
Lactose in water containing 14 per cent sucrose....................	50	12.63	13.93	12.63	13.93
Lactose in water containing 14 per cent sucrose....................	65	14.95	17.60	15.16	17.88
Lactose in whole milk containing 14 per cent sucrose.............	65	14.77	17.30	15.25	17.98
Lactose in skim milk containing 14 per cent sucrose.............	65	14.70	17.30	14.89	17.46
Lactose in water containing 62 per cent sucrose................	65	13.20	15.20	13.46	15.55
Lactose in milk containing 62 per cent sucrose....................	65	13.45	15.50	13.45	15.50
Lactose in supersaturated sucrose-water solution..................	65	14.49	16.94		
Lactose in supersaturated sucrose-milk solution..................	65	14.01	16.30		
Lactose in water (by Hudson)....	50	13.06	15.02		
Lactose in water (by Hudson)....	65	15.54	18.40		

* The results given in the second and third columns of the table are considered somewhat more accurate and were obtained after the method of procedure had become well established. However, the averages of all results as given in columns four and five of the table are not seriously at variance with former figures and with those by Hudson.

from above, i.e., from undersaturation by adding the dry lactose to the cold liquid and allowing it to dissolve, to saturation, and from supersaturation by dissolving the dry lactose in the heated

liquid, cooling and allowing to crystallize until equilibrium was reached. The results of the two procedures checked closely, indicating that the point of equilibrium had actually been reached.

4. Lactose solubility was determined by: first, noting the maximum amount of lactose which will dissolve in or crystallize out from prearranged series of solutions of graduated concentrations; second, total solids determinations of the filtered solutions; and third, the gravimetric Fehling's solution method. The results obtained by the different methods were in close agreement.

5. Averages of all determinations of lactose solubility for each type of solution used are given in table 8.

CONCLUSIONS

The results on lactose solubility suggest the following deductions:

1. That the colloids in milk do not have any material influence on the solubility of lactose.

2. That the presence of sucrose diminishes the solubility of lactose somewhat. This decrease is only slight in dilute sucrose solutions such as are comparable to ice cream mix, but becomes greater as the concentration of sucrose increases, the decrease in sucrose solutions, comparable to sweetened condensed milk, amounting to approximately 15 per cent of the lactose solubility in aqueous solutions.

3. It is of interest to point out here, that the presence in lactose solutions of high sucrose concentration also has a very noticeable influence on lactose crystal formation, the shape of the lactose crystals being distinctly modified. The lactose crystals from such solutions are short, blunt and apparently do not assume the typical form of lactose crystals from supersaturated solutions of lactose in water, as described and illustrated in Part II on "Lactose crystal formation" which will appear in the next issue of this JOURNAL.

Our present knowledge of this subject is too limited to justify any discussion as to the possible relation between the effect of

sucrose on lactose solubility and on the form of lactose crystals. However, these observations are suggestive of the possibility that such a relation may exist.

A similar parallel is observed between the effect of milk colloids on lactose solubility and on the form of lactose crystals, in that the milk colloids appear to have no effect in either case, as also shown in Part II on "Lactose crystal formation."

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ABSTRACTS OF FOREIGN DAIRY LITERATURE

J. L. HILEMAN

BLANCHETIERE. *Seasonal Variations of Some Mineral Elements of Milk.* (C. R. des seances de la Soc. de Biol. (France), Vol. 92, p. 1295, 1925. Abstracted in Le Lait, Vol. 6, April, 1926, p. 290.)

In mixed milk furnished to the hospitals of Lens, the alkali metals sodium and potassium diminish in amount in winter, while the alkaline earth metal calcium increases in winter. This cannot be explained by any variation in the content of these elements in the food, nor by the theory of Taylor and Usband that there is an inverse relation between the composition of the milk and the rapidity of secretion, since the different elements do not vary in the same direction.

The author explains the phenomena by the fact that the vitamin A, which is ingested in greater quantity in summer, causes a greater retention of calcium in the organism, as shown, among others, by Hart, Steenbock and Hoppert, thus leaving less available for the milk.

F. RICHARD. *Analysis of Milk Powder.* (Le Lait, Vol. 6, February, 1926, p. 86.)

In a series of comparisons, the Röse-Gottlieb method for fat in milk powder gave consistently lower results (from 0.60 to 1.90 per cent difference) than the Weibull method. (See abstract of article by Schoonjans.)

Moisture is determined in milk powder by heating five grams of powder in a tarred shallow dish at 100°C., cooling in a desiccator, weighing, and taking the loss in weight as moisture.

ALBERT SCHOONJANS. *Note on the Determination of Fat in Milk Powders.* (Le Lait, Vol. 5, October, 1925, p. 782.)

Schoonjans describes and compares results with three methods of determining fat in milk powder; the common Röse-Gottlieb, the Weibull and the Teichert.

In the Weibull method, 2.5 grams of powder are boiled in a flask for fifteen minutes with 30 cc. of eighth-normal hydrochloric acid and 20 cc. of water, adding a small amount of pumice stone to avoid bumping. The solution is then filtered while hot through a moist

paper and the residue on the filter paper is washed till free from acid. The filter paper and its contents are dried at 100°C. and extracted in a Soxhlet apparatus to determine the amount of fat. This method gives consistently higher results than the Röse-Gottlieb method, the difference between the two varying from 0.15 to 1.30 per cent.

The Teichert method is a modification of the Gerber method. Place 2.5 grams of powder, 10 cc. of sulphuric acid (specific gravity 1.81) and 1 cc. of amyl alcohol in a tube made especially for the purpose, shake, centrifuge and read the percentage of fat as in the Gerber method. This method gives results only slightly higher (from 0.05 to 0.15 per cent) than the Weibull method.

Tschirch and Barben. *Rancidity of Fats.* (Schweiz Apothekerztg., Vol. 62, p. 281, 1924. Abstracted in Le Lait, Vol. 6, February, 1926, p. 119.)

In order for a fat to become rancid, there must be present water, air, light, and an unsaturated fatty acid. Exposed to air and moisture, unsaturated molecule adds oxygen at each double bond, forming a peroxide. Water eliminates a part of that oxygen with the formation of an oxide, together with hydrogen peroxide and ozone. The ozone gives rise to the formation of less stable ozonides, which are split by the water into simpler molecules of odorous aldehydes, ketones and acids.

By imitating the above conditions with lead peroxide (PbO_2) and sulphuric acid, the author produced rancidity in peanut oil. In the rancid fat, one can easily detect hydrogen peroxide: it gives the Kreis reaction.

This author apparently uses the term rancid in the sense in which we use tallowy, since the fat was described as having the odor of tallow.

Weigmann and Todt. *Experiments on the Manufacture of Cheese with Pasteurized Milk.* (Molkerei Zeitung (Hildesheim), Nos. 46, 47, and 48, June, 1923. Abstracted in Le Lait, Vol. 6, February, 1926, p. 130).

Milk was heated at 110°C. and made into cheese after adding calcium chloride and a lactic starter. The rennet acted normally and the curd was malleable under pressure, but the cheese did not ripen properly and was worthless.

Satisfactory cheese can be made from milk heated to from 95° to

97°C., and also from milk held for thirty minutes at 63°C. The former is less open in texture.

Pasteurization retards ripening, and the cheese from pasteurized milk has a smoother rind and is less subject to mold growth than cheese from unpasteurized milk.

L. LINDET. *On the Coagulation of Casein in the Presence of Salts of Lime in Acid Solution.* (Le Lait, Vol. 5, December, 1925, p. 953.)

Casein precipitated from skim milk by lactic acid or other acids and treated with an excess of acid forms a jelly-like solution. The casein can be reprecipitated from this solution by the addition of calcium salts. The reprecipitated casein contains approximately the same amount of phosphoric acid as the original precipitate (about 2 per cent P_2O_5) but almost no lime, whereas the original precipitate contained from 0.89 to 1.5 per cent CaO.

The author draws the conclusion that acid alone is not capable of bringing about the coagulation of casein, but that the presence of calcium salts in solution is necessary. The data presented in support of this conclusion are rather meager.

W. DORNER. *A Simple Procedure for the Staining of Spores.* (Le Lait, Vol. 6, January, 1926, p. 8.)

This method is a combination of the negative staining method of Burri, in which the bacteria are not stained but are surrounded by a black background of India ink or nigrosine B, and of Klein's method of staining spores. The nigrosine B takes the place of alcohol in decolorizing the bacterial cells after staining with carbol fuchsin, leaving the spores a bright red, the bacterial cells surrounding the spore colorless, and the background black.

The technique of the method is as follows:

1. Prepare a suspension of the bacteria to be stained in 0.5 cc. of water in a centrifuge tube.

2. Add 1 to 2 cc. of a melted 10 per cent gelatin solution.

3. Add 1 to 2 cc. of carbol fuchsin solution.

4. Heat the tube in a boiling water-bath for ten minutes.

5. Wash the bacteria to remove the gelatin. To do this, add 10 to 15 cc. of water, and centrifuge for some minutes, or allow to settle for several hours.

6. Mix a loop of the sediment from the bottom of the tube with a loop of a saturated aqueous solution of nigrosine B on a slide and dry.

7. Examine with an oil-immersion lens.

BLEYER AND KALLMAN. *Contribution to the Knowledge of Some Little-Studied Components of Milk.* (Biochemische Zeitschrift, 153, Nos. 3–6, pp. 459. Abstracted in Le Lait, Vol. 6, January, 1926, p. 26.)

These authors find that the nitrogenous bodies in milk are casein, albumin (globulin and pseudoglobulin), albumose, peptone, and small amounts of purines, uric acid, urea, ammonia, creatine, creatinine, amino acids, sulphocyanide, and a nitrogenous coloring matter.

HAGLUND, BARTHEL AND SANDBERG. *The Number of Bacteria in Milk for Cheese-Making and the Rapidity of Ripening of the Cheese.* (Communication No. 270, Central Agricultural Experiment Station. Bacteriological Section No. 35, Stockholm, 1924. Abstracted in Le Lait, Vol. 6, January, 1926, p. 31.)

Increasing either the number of bacteria or the acidity of the milk independently at the time of coagulation increases the rapidity of the ripening of the cheese, as measured by the quantity of soluble nitrogen compounds formed during a certain period of time.

DAIRY NOTES

The following information in regard to the plans to hold an International Dairy Congress in England in 1928, has been taken from recent numbers of "The Milk Industry" published in London.

Preparations for the Congress are proceeding apace. Meetings of the General Committee were held in April and in June. Mr. R. Ravenscroft, Secretary of the British Dairy Farmers' Association, 28 Russell Square, London, S. W. 1, England, is acting as Secretary for the Congress and is working with the greatest enthusiasm to make the Congress a success. A provisional committee has worked out a tentative program with estimates of the cost and has satisfied the Executive Committee as to the practicability of holding the Congress.

It is proposed that the Congress should cover a period of ten days, three of which would be spent in London, two in Reading, two at the Royal Agricultural Society's Show to be held at Warwick and the remaining three days to be spent in Scotland. In order to include the Royal Show it will be necessary to hold the Congress during the first week of July. Lord Kenyon has accepted the Presidency and extensive preparations are being made for the entertainment of visiting delegates.

Among the subjects recommended for papers are the breeding and feeding of animals for milk production, milk transportation, milk distribution, organization for advisory assistance, dairy research, dairy machinery and equipment, international questions and coöperation.

The placing of the Congress during the early part of the summer will make it necessary for those who go directly from North America to the Congress to travel at the height of the tourist season. For this reason plans must be worked out a long time in advance. Preliminary inquiries are already being made to determine the number of Americans who are likely to go, the thought being that a group might like to join in securing space on the same steamer.

<div align="right">R. S. BREED.</div>

CONNECTICUT. Prof. George C. White, head of the Dairy Department and Dean of the College of Agriculture, has been at Ithaca, N. Y.,

since September 1. His leave of absence carries through to the first of February, 1927. He is pursuing special studies in animal diseases and in the field of education at Cornell University. Professor White is secretary of the American Dairy Science Association and he may be addressed care of the Department of Dairy Industry at Cornell University.

MASSACHUSETTS. Mr. Richard Smith, Jr., has returned to the dairy staff after a year's leave of absence during which time he did graduate study in the dairy husbandry department of the University of Illinois.

NEW YORK. Mr. J. C. Marquardt of the Experiment Station at Geneva, N. Y. has recently returned from Cornell University where he has completed the requirements for the degree of Master of Science. Mr. J. C. Hening will study at the same institution for an advanced degree this college year.

NEW YORK, CORNELL COLLEGE. Prof. H. E. Ross has recently returned from his sabbatic leave of absence of one year, which he spent in Argentina aiding the Government in the improvement of the city milk supply and in the installation of modified milk laboratories.

Prof. J. D. Brew is on sabbatic leave for one year. He is doing graduate work at the University of California.

Prof. W. W. Fisk is on a leave of absence for one and one-half years during which time he will be engaged in commercial activities.

Dr. Arthur T. Henrici, Professor of Bacteriology and Immunology of the Medical College of the University of Minnesota, has been appointed acting Professor for the year 1926–27. During this period Dr. Henrici will be on sabbatic leave from the University of Minnesota.

Dr. Phil. Karl Demeter, head of the Bacteriological Department in the South German Research Institute for Dairy Science, Weihenstephan, near Munich, Germany, will sail for home on October 14. Dr. Demeter has been doing special research in the department of dairy industry, and is one of many young scientists who have had opportunity to come to America under the auspices of the International Education Board.

Dr. Norman C. Wright, assistant research chemist at the National Institute for Research in Dairying at the University of Reading, Reading, England, has just arrived at Cornell University for two years of research study in the department of dairy industry. Dr. Wright is a

fellow on the Commonwealth Fund. He will have opportunity during his two years of study in America to travel three months each year.

Other recent appointments include Georges Knaysi, Instructor in Bacteriology; Walter Hochstrasser, Instructor in Dairy Industry, and G. M. Bateman, Instructor in Dairy Chemistry.

ERRATUM

In the September number of the JOURNAL OF DAIRY SCIENCE the chart on page 486 should be on page 485 with the legend—"Fig. 1. Effect of heating at 101.5°C. on the hydrogen-ion concentration and on the titratable acidity of milk." The chart on page 485 should be on page 486 with the legend—"Fig. 2. Effect of heating at 95°C. on the hydrogen-ion concentration and on the titratable acidity of milk."

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